

PATENT APPLICATION

**INDUCING CELLULAR IMMUNE RESPONSES TO p53 USING PEPTIDE AND
NUCLEIC ACID COMPOSITIONS**

Inventor(s): John Fikes, a United States citizen, residing at
6494 Lipmann Street
San Diego, California 92122

Alessandro Sette, an Italian citizen, residing at
5551 Linda Rosa Avenue
La Jolla, California 92037

John Sidney, a United States citizen, residing at
4218 Corte de la Siena
San Diego, California 92130

Scott Southwood, a United States citizen, residing at
10679 Strathmore Drive
Santee, California 92071

Robert Chesnut, a United States citizen, residing at
1473 Kings Cross Drive
Cardiff-by-the-Sea, California 92007

Esteban Celis, a United States citizen, residing at
3683 Wright Road S.W.
Rochester, Minnesota 55902

Elissa Keogh, a United States citizen, residing at
4343 Caminito del Diamante
San Diego, California 92121

5 **INDUCING CELLULAR IMMUNE RESPONSES TO p53 USING PEPTIDE AND
NUCLEIC ACID COMPOSITIONS**

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a Continuation-In-Part ("CIP") of U.S.S.N. 09/189,702, filed 11/10/98, which is a CIP of U.S.S.N 08/205,713 filed 3/4/94, which is a CIP of abandoned
10 U.S.S.N. 08/159,184 filed 11/29/93, which is a CIP of abandoned U.S.S.N. 08/073,205 filed 6/4/93 which is a CIP of abandoned U.S.S.N 08/027,146 filed 3/5/93. The present application is also related to U.S.S.N. 09/226,775, which is a CIP of abandoned U.S.S.N. 08/815,396, which claims benefit of abandoned U.S.S.N. 60/013,113. Furthermore, the present application is related to U.S.S.N. 09/017,735, which is a CIP of abandoned U.S.S.N.
15 08/589,108; U.S.S.N. 08/454,033; and U.S.S.N. 08/349,177. The present application is also related to U.S.S.N. 09/017,524, U.S.S.N. 08/821,739, which claims benefit of abandoned U.S.S.N. 60/013,833; and U.S.S.N. 08/347,610, which is a CIP of U.S.S.N. 08/159,339, which is a CIP of abandoned U.S.S.N. 08/103,396, which is a CIP of abandoned U.S.S.N. 08/027,746, which is a CIP of abandoned U.S.S.N. 07/926,666. The present application is
20 also related to U.S.S.N. 09/017,743, which is a CIP of abandoned U.S.S.N. 08/590,298; and U.S.S.N. 08/452,843, which is a CIP of U.S.S.N. 08/344,824, which is a CIP of abandoned U.S.S.N. 08/278,634. The present application is also related to PCT application 99/12066 filed 5/28/99 which claims benefit of provisional U.S.S.N. 60/087,192, and U.S.S.N. 09/009,953, which is a CIP of abandoned U.S.S.N. 60/036,713 and abandoned U.S.S.N.
25 60/037,432. In addition, the present application is related to U.S.S.N. 09/098,584, U.S.S.N. 09/239,043, U.S.S.N. 60/117,486, U.S.S.N. 09/350,401, and U.S.S.N. 09/357,737. In addition, the present application is related to U.S. Patent Application entitled "Inducing Cellular Immune Responses to Carcinoembryonic Antigen Using Peptide and Nucleic Acid Compositions", Attorney Docket No. 018623-014400, filed of even date herewith; U.S.
30 Patent Application entitled "Inducing Cellular Immune Responses to MAGE2/3 Using Peptide and Nucleic Acid Compositions", Attorney Docket No. 018623-014600, filed of even date herewith; and U.S. Patent Application entitled "Inducing Cellular Immune Responses to HER2/neu Using Peptide and Nucleic Acid Compositions", Attorney Docket

09458297 " 121099

No. 018623-014800, filed of even date herewith. All of the above applications are incorporated herein by reference.

FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

- 5 This invention was funded, in part, by the United States government under grants with the National Institutes of Health. The U.S. government has certain rights in this invention.

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INDEX

	I.	Background of the Invention
	II.	Summary of the Invention
	III.	Brief Description of the Figures
5	IV.	Detailed Description of the Invention
	A.	Definitions
	B.	Stimulation of CTL and HTL responses
	C.	Binding Affinity of Peptide Epitopes for HLA Molecules
	D.	Peptide Epitope Binding Motifs and Supermotifs
10	1.	HLA-A1 supermotif
	2.	HLA-A2 supermotif
	3.	HLA-A3 supermotif
	4.	HLA-A24 supermotif
	5.	HLA-B7 supermotif
15	6.	HLA-B27 supermotif
	7.	HLA-B44 supermotif
	8.	HLA-B58 supermotif
	9.	HLA-B62 supermotif
20	10.	HLA-A1 motif
	11.	HLA-A2.1 motif
	12.	HLA-A3 motif
	13.	HLA-A11 motif
	14.	HLA-A24 motif
	15.	HLA-DR-1-4-7 supermotif
25	16.	HLA-DR3 motifs
	E.	Enhancing Population Coverage of the Vaccine
	F.	Immune Response-Stimulating Peptide Epitope Analogs
	G.	Computer Screening of Protein Sequences from Disease-Related Antigens for Supermotif- or Motif-Containing Epitopes
30	H.	Preparation of Peptide Epitopes

660727 26285460

- I. Assays to Detect T-Cell Responses
- J. Use of Peptide Epitopes for Evaluating Immune Responses
- K. Vaccine Compositions
 - 1. Minigene Vaccines
 - 5 2. Combinations of CTL Peptides with Helper Peptides
- L. Administration of Vaccines for Therapeutic or Prophylactic Purposes
- M. Kits
- V. Examples
- VI. Claims
- 10 VII. Abstract

09458297 121099
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I. BACKGROUND OF THE INVENTION

A growing body of evidence suggests that cytotoxic T lymphocytes (CTL) are important in the immune response to tumor cells. CTL recognize peptide epitopes in the context of HLA class I molecules that are expressed on the surface of almost all nucleated cells. Following intracellular processing of endogenously synthesized tumor antigens, antigen-derived peptide epitopes bind to class I HLA molecules in the endoplasmic reticulum, and the resulting complex is then transported to the cell surface. CTL recognize the peptide-HLA class I complex, which then results in the destruction of the cell bearing the HLA-peptide complex directly by the CTL and/or via the activation of non-destructive mechanisms, *e.g.*, activation of lymphokines such as tumor necrosis factor- α (TNF- α) or interferon- γ (IFN γ) which enhance the immune response and facilitate the destruction of the tumor cell.

Tumor-specific helper T lymphocytes (HTLs) are also known to be important for maintaining effective antitumor immunity. Their role in antitumor immunity has been demonstrated in animal models in which these cells not only serve to provide help for induction of CTL and antibody responses, but also provide effector functions, which are mediated by direct cell contact and also by secretion of lymphokines (*e.g.*, IFN γ and TNF- α).

A fundamental challenge in the development of an efficacious tumor vaccine is immune suppression or tolerance that can occur. There is therefore a need to establish vaccine embodiments that elicit immune responses of sufficient breadth and vigor to prevent progression and/or clear the tumor.

The epitope approach, as we have described, may represent a solution to this challenge, in that it allows the incorporation of various antibody, CTL and HTL epitopes, from discrete regions of a target TAA, and/or regions of other TAAs, in a single vaccine composition. Such a composition may simultaneously target multiple dominant and subdominant epitopes and thereby be used to achieve effective immunization in a diverse population.

The p53 protein is normally a tumor suppressor gene that, in normal cells, induces cell cycle arrest which allows DNA to be monitored for irregularities and maintains DNA integrity (*see, e.g.*, Kuerbitz *et al.*, *Proc. Natl. Acad. Sci USA* 89:7491-7495, 1992). Mutations in the gene abolish its suppressor function and result in escape from controlled growth. The most common mutations are at positions 175, 248, 273, and 282 and have

09458297 121099

been observed in colon (Rodrigues *et al.*, *Proc. Natl. Acad. Sci. USA* 87:7555-7559, 1990), lung (Fujino *et al.*, *Cancer* 76:2457-2463, 1995), prostate (Eastham *et al.*, *Clin. Cancer Res.* 1:1111-1118, 1995), bladder (Vet *et al.*, *Lab. Invest.* 73:837-843, 1995) and osteosarcomas (Abudu *et al.*, *Br. J. Cancer* 79:1185-1189, 1999; Hung *et al.*, *Acta Orthop. Scand. Supp.* 273:68-73, 1997).

The mutations in p53 also lead to overexpression of both the wildtype and mutated p53 (*see, e.g.*, Levine *et al.*, *Nature* 351:453-456, 1991) thereby making it more likely that epitopes within the protein may be recognized by the immune system. Thus, p53 is an important target for cellular immunotherapy.

The information provided in this section is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this application. Accordingly, information in this section is not intended, in any way, to delineate the priority date for the invention.

II. SUMMARY OF THE INVENTION

This invention applies our knowledge of the mechanisms by which antigen is recognized by T cells, for example, to develop epitope-based vaccines directed towards TAAs. More specifically, this application communicates our discovery of specific epitope pharmaceutical compositions and methods of use in the prevention and treatment of cancer.

Upon development of appropriate technology, the use of epitope-based vaccines has several advantages over current vaccines, particularly when compared to the use of whole antigens in vaccine compositions. For example, immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines. Such immunosuppressive epitopes may, *e.g.*, correspond to immunodominant epitopes in whole antigens, which may be avoided by selecting peptide epitopes from non-dominant regions (*see, e.g.*, Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996).

An additional advantage of an epitope-based vaccine approach is the ability to combine selected epitopes (CTL and HTL), and further, to modify the composition of the epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

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Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by infectious agents or whole protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune
 5 response to multiple selected antigens from the same pathogen (a "pathogen" may be an infectious agent or a tumor-associated molecule). Thus, patient-by-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes from multiple antigens from the pathogen in a vaccine composition.

Furthermore, an epitope-based anti-tumor vaccine also provides the opportunity to
 10 combine epitopes derived from multiple tumor-associated molecules. This capability can therefore address the problem of tumor-to tumor variability that arises when developing a broadly targeted anti-tumor vaccine for a given tumor type and can also reduce the likelihood of tumor escape due to antigen loss. For example, a breast cancer tumor in one patient may express a target TAA that differs from a breast cancer tumor in another
 15 patient. Epitopes derived from multiple TAAs can be included in a polyepitopic vaccine that will target both breast cancer tumors.

One of the most formidable obstacles to the development of broadly efficacious epitope-based immunotherapeutics, however, has been the extreme polymorphism of HLA molecules. To date, effective non-genetically biased coverage of a population has
 20 been a task of considerable complexity; such coverage has required that epitopes be used that are specific for HLA molecules corresponding to each individual HLA allele. Impractically large numbers of epitopes would therefore have to be used in order to cover ethnically diverse populations. Thus, there has existed a need for peptide epitopes that are bound by multiple HLA antigen molecules for use in epitope-based vaccines. The
 25 greater the number of HLA antigen molecules bound, the greater the breadth of population coverage by the vaccine.

Furthermore, as described herein in greater detail, a need has existed to modulate peptide binding properties, *e.g.*, so that peptides that are able to bind to multiple HLA molecules do so with an affinity that will stimulate an immune response. Identification of
 30 epitopes restricted by more than one HLA allele at an affinity that correlates with immunogenicity is important to provide thorough population coverage, and to allow the elicitation of responses of sufficient vigor to prevent or clear an infection in a diverse segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

"PATENT" 2025-10-10

In a preferred embodiment, epitopes for inclusion in vaccine compositions of the invention are selected by a process whereby protein sequences of known antigens are evaluated for the presence of motif or supermotif-bearing epitopes. Peptides corresponding to a motif- or supermotif-bearing epitope are then synthesized and tested for the ability to bind to the HLA molecule that recognizes the selected motif. Those peptides that bind at an intermediate or high affinity *i.e.*, an IC_{50} (or a K_D value) of 500 nM or less for HLA class I molecules or an IC_{50} of 1000 nM or less for HLA class II molecules, are further evaluated for their ability to induce a CTL or HTL response. Immunogenic peptide epitopes are selected for inclusion in vaccine compositions.

Supermotif-bearing peptides may additionally be tested for the ability to bind to multiple alleles within the HLA supertype family. Moreover, peptide epitopes may be analogued to modify binding affinity and/or the ability to bind to multiple alleles within an HLA supertype.

The invention also includes embodiments comprising methods for monitoring or evaluating an immune response to a TAA in a patient having a known HLA-type. Such methods comprise incubating a T lymphocyte sample from the patient with a peptide composition comprising a TAA epitope that has an amino acid sequence described in Tables VII to Table XX or Table XXII which binds the product of at least one HLA allele present in the patient, and detecting for the presence of a T lymphocyte that binds to the peptide. A CTL peptide epitope may, for example, be used as a component of a tetrameric complex for this type of analysis.

An alternative modality for defining the peptide epitopes in accordance with the invention is to recite the physical properties, such as length; primary structure; or charge, which are correlated with binding to a particular allele-specific HLA molecule or group of allele-specific HLA molecules. A further modality for defining peptide epitopes is to recite the physical properties of an HLA binding pocket, or properties shared by several allele-specific HLA binding pockets (*e.g.* pocket configuration and charge distribution) and reciting that the peptide epitope fits and binds to the pocket or pockets.

As will be apparent from the discussion below, other methods and embodiments are also contemplated. Further, novel synthetic peptides produced by any of the methods described herein are also part of the invention.

III. BRIEF DESCRIPTION OF THE FIGURES

not applicable

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IV. DETAILED DESCRIPTION OF THE INVENTION

The peptide epitopes and corresponding nucleic acid compositions of the present invention are useful for stimulating an immune response to a TAA by stimulating the production of CTL or HTL responses. The peptide epitopes, which are derived directly or indirectly from native TAA protein amino acid sequences, are able to bind to HLA molecules and stimulate an immune response to the TAA. The complete sequence of the TAA proteins to be analyzed can be obtained from GenBank. Peptide epitopes and analogs thereof can also be readily determined from sequence information that may subsequently be discovered for heretofore unknown variants of particular TAAs, as will be clear from the disclosure provided below.

A list of target TAA includes, but is not limited to, the following antigens: MAGE 1, MAGE 2, MAGE 3, MAGE-11, MAGE-A10, BAGE, GAGE, RAGE, MAGE-C1, LAGE-1, CAG-3, DAM, MUC1, MUC2, MUC18, NY-ESO-1, MUM-1, CDK4, BRCA2, NY-LU-1, NY-LU-7, NY-LU-12, CASP8, RAS, KIAA-2-5, SCCs, p53, p73, CEA, Her 2/neu, Melan-A, gp100, tyrosinase, TRP2, gp75/TRP1, kallikrein, PSM, PAP, PSA, PT1-1, B-catenin, PRAME, Telomerase, FAK, cyclin D1 protein, NOEY2, EGF-R, SART-1, CAPB, HPVE7, p15, Folate receptor CDC27, PAGE-1, and PAGE-4.

The peptide epitopes of the invention have been identified in a number of ways, as will be discussed below. Also discussed in greater detail is that analog peptides have been derived and the binding activity for HLA molecules modulated by modifying specific amino acid residues to create peptide analogs exhibiting altered immunogenicity. Further, the present invention provides compositions and combinations of compositions that enable epitope-based vaccines that are capable of interacting with HLA molecules encoded by various genetic alleles to provide broader population coverage than prior vaccines.

IV.A. Definitions

The invention can be better understood with reference to the following definitions, which are listed alphabetically:

A "computer" or "computer system" generally includes: a processor; at least one information storage/retrieval apparatus such as, for example, a hard drive, a disk drive or a tape drive; at least one input apparatus such as, for example, a keyboard, a mouse, a touch screen, or a microphone; and display structure. Additionally, the computer may

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include a communication channel in communication with a network. Such a computer may include more or less than what is listed above.

"Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

5 A "cryptic epitope" elicits a response by immunization with an isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein which comprises the epitope is used as an antigen.

A "dominant epitope" is an epitope that induces an immune response upon immunization with a whole native antigen (see, e.g., Sercarz, *et al.*, *Annu. Rev. Immunol.* 10 11:729-766, 1993). Such a response is cross-reactive *in vitro* with an isolated peptide epitope.

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system 15 setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule. Throughout this disclosure epitope and peptide are often used interchangeably. It is to be appreciated, 20 however, that isolated or purified protein or peptide molecules larger than and comprising an epitope of the invention are still within the bounds of the invention.

"Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (see, e.g., Stites, *et al.*, *IMMUNOLOGY*, 8TH ED., Lange Publishing, Los Altos, CA, 1994).

25 An "HLA supertype or family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like molecules (where xx denotes a particular HLA type), are 30 synonyms.

Throughout this disclosure, results are expressed in terms of "IC₅₀'s." IC₅₀ is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_D

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values. Assays for determining binding are described in detail, e.g., in PCT publications WO 94/20127 and WO 94/03205. It should be noted that IC₅₀ values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (e.g., HLA preparation, etc.). For example, excessive concentrations of HLA

5 molecules will increase the apparent measured IC₅₀ of a given ligand.

Alternatively, binding is expressed relative to a reference peptide. Although as a particular assay becomes more, or less, sensitive, the IC₅₀'s of the peptides tested may change somewhat, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC₅₀ of the reference

10 peptide increases 10-fold, the IC₅₀ values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC₅₀, relative to the IC₅₀ of a standard peptide.

Binding may also be determined using other assay systems including those using:

15 live cells (e.g., Ceppellini *et al.*, *Nature* 339:392, 1989; Christnick *et al.*, *Nature* 352:67, 1991; Busch *et al.*, *Int. Immunol.* 2:443, 1990; Hill *et al.*, *J. Immunol.* 147:189, 1991; del Guercio *et al.*, *J. Immunol.* 154:685, 1995), cell free systems using detergent lysates (e.g., Cerundolo *et al.*, *J. Immunol.* 21:2069, 1991), immobilized purified MHC (e.g., Hill *et al.*, *J. Immunol.* 152, 2890, 1994; Marshall *et al.*, *J. Immunol.* 152:4946, 1994), ELISA

20 systems (e.g., Reay *et al.*, *EMBO J.* 11:2829, 1992), surface plasmon resonance (e.g., Khilko *et al.*, *J. Biol. Chem.* 268:15425, 1993); high flux soluble phase assays (Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994), and measurement of class I MHC stabilization or assembly (e.g., Ljunggren *et al.*, *Nature* 346:476, 1990; Schumacher *et al.*, *Cell* 62:563, 1990; Townsend *et al.*, *Cell* 62:285, 1990; Parker *et al.*, *J. Immunol.* 149:1896, 1992).

25 As used herein, "high affinity" with respect to HLA class I molecules is defined as binding with an IC₅₀, or K_D value, of 50 nM or less; "intermediate affinity" is binding with an IC₅₀ or K_D value of between about 50 and about 500 nM. "High affinity" with respect to binding to HLA class II molecules is defined as binding with an IC₅₀ or K_D value of 100 nM or less; "intermediate affinity" is binding with an IC₅₀ or K_D value of

30 between about 100 and about 1000 nM.

The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues that are the same, when compared and

aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

An "immunogenic peptide" or "peptide epitope" is a peptide that comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and induce a CTL and/or HTL response. Thus, immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and thereafter inducing a cytotoxic T cell response, or a helper T cell response, to the antigen from which the immunogenic peptide is derived.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment.

"Major Histocompatibility Complex" or "MHC" is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see, Paul, FUNDAMENTAL IMMUNOLOGY, 3RD ED., Raven Press, New York, 1993.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A "negative binding residue" or "deleterious residue" is an amino acid which, if present at certain positions (typically not primary anchor positions) in a peptide epitope, results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule.

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α -amino and carboxyl groups of adjacent amino acids. The preferred CTL-inducing peptides of the invention are 13 residues or less in length and usually consist of between about 8 and about 11 residues, preferably 9 or 10 residues. The preferred HTL-inducing oligopeptides are less than

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about 50 residues in length and usually consist of between about 6 and about 30 residues, more usually between about 12 and 25, and often between about 15 and 20 residues.

"Pharmaceutically acceptable" refers to a non-toxic, inert, and/or physiologically compatible composition.

5 A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding
10 grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. In one embodiment, for example, the primary anchor residues are located at position 2 (from the amino terminal position) and at the carboxyl terminal position of a 9-residue peptide epitope in accordance with the invention. The primary anchor positions for each motif and supermotif are set forth in Table 1. For
15 example, analog peptides can be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide comprising a particular motif or supermotif.

 "Promiscuous recognition" is where a distinct peptide is recognized by the same T cell clone in the context of various HLA molecules. Promiscuous recognition or binding
20 is synonymous with cross-reactive binding.

 A "protective immune response" or "therapeutic immune response" refers to a CTL and/or an HTL response to an antigen derived from an infectious agent or a tumor antigen, which prevents or at least partially arrests disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by
25 the stimulation of helper T cells.

 The term "residue" refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

 A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor
30 residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or intermediate affinity binding peptides, or a residue otherwise associated with high or

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intermediate affinity binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

5 A "subdominant epitope" is an epitope which evokes little or no response upon immunization with whole antigens which comprise the epitope, but for which a response can be obtained by immunization with an isolated peptide, and this response (unlike the case of cryptic epitopes) is detected when whole protein is used to recall the response *in vitro* or *in vivo*.

10 A "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. Preferably, a supermotif-bearing peptide is recognized with high or intermediate affinity (as defined herein) by two or more HLA molecules.

"Synthetic peptide" refers to a peptide that is not naturally occurring, but is man-made using such methods as chemical synthesis or recombinant DNA technology.

15 The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the position closest to the amino terminal end of the epitope, or the peptide or protein of which it may be a part. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G. Symbols for the amino acids are shown below.

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Single Letter Symbol	Three Letter Symbol	Amino Acids
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic Acid
E	Glu	Glutamic Acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

IV.B. Stimulation of CTL and HTL responses

The mechanism by which T cells recognize antigens has been delineated during the past ten years. Based on our understanding of the immune system we have developed efficacious peptide epitope vaccine compositions that can induce a therapeutic or prophylactic immune response to a TAA in a broad population. For an understanding of the value and efficacy of the claimed compositions, a brief review of immunology-related technology is provided.

A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al.*, *Cell* 47:1071, 1986; Babbitt, B. P. *et al.*, *Nature* 317:359, 1985; Townsend, A. and Bodmer, H., *Annu. Rev. Immunol.* 7:601,

1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to HLA antigen molecules have been identified and are described herein and are set forth in Tables I, II, and III (*see also, e.g., Southwood, et al., J. Immunol.* 160:3363, 1998; Rammensee, *et al., Immunogenetics* 41:178, 1995; Rammensee *et al., SYFPEITHI*, access via web at : <http://134.2.96.221/scripts.hlaserver.dll/home.htm>; Sette, A. and Sidney, J. *Curr. Opin. Immunol.* 10:478, 1998; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, J. *Curr. Biol.* 6:52, 1994; Ruppert *et al., Cell* 74:929-937, 1993; Kondo *et al., J. Immunol.* 155:4307-4312, 1995; Sidney *et al., J. Immunol.* 157:3480-3490, 1996; Sidney *et al., Human Immunol.* 45:79-93, 1996; Sette, A. and Sidney, J. *Immunogenetics*, in press, 1999).

Furthermore, x-ray crystallographic analysis of HLA-peptide complexes has revealed pockets within the peptide binding cleft of HLA molecules which accommodate, in an allele-specific mode, residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present. (*See, e.g., Madden, D.R. Annu. Rev. Immunol.* 13:587, 1995; Smith, *et al., Immunity* 4:203, 1996; Fremont *et al., Immunity* 8:305, 1998; Stern *et al., Structure* 2:245, 1994; Jones, E.Y. *Curr. Opin. Immunol.* 9:75, 1997; Brown, J. H. *et al., Nature* 364:33, 1993; Guo, H. C. *et al., Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al., Nature* 360:364, 1992; Silver, M. L. *et al., Nature* 360:367, 1992; Matsumura, M. *et al., Science* 257:927, 1992; Madden *et al., Cell* 70:1035, 1992; Fremont, D. H. *et al., Science* 257:919, 1992; Saper, M. A., Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991.)

Accordingly, the definition of class I and class II allele-specific HLA binding motifs, or class I or class II supermotifs allows identification of regions within a protein that have the potential of binding particular HLA molecules.

The present inventors have found that the correlation of binding affinity with immunogenicity, which is disclosed herein, is an important factor to be considered when evaluating candidate peptides. Thus, by a combination of motif searches and HLA-peptide binding assays, candidates for epitope-based vaccines have been identified. After determining their binding affinity, additional confirmatory work can be performed to select, amongst these vaccine candidates, epitopes with preferred characteristics in terms of population coverage, antigenicity, and immunogenicity.

Various strategies can be utilized to evaluate immunogenicity, including:

- 1) Evaluation of primary T cell cultures from normal individuals (*see, e.g.,* Wentworth, P. A. *et al.*, *Mol. Immunol.* 32:603, 1995; Celis, E. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al.*, *J. Immunol.* 158:1796, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998); This procedure involves the stimulation of peripheral blood lymphocytes (PBL) from normal subjects with a test peptide in the presence of antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using, *e.g.*, a ^{51}Cr -release assay involving peptide sensitized target cells.
- 2) Immunization of HLA transgenic mice (*see, e.g.,* Wentworth, P. A. *et al.*, *J. Immunol.* 26:97, 1996; Wentworth, P. A. *et al.*, *Int. Immunol.* 8:651, 1996; Alexander, J. *et al.*, *J. Immunol.* 159:4753, 1997); In this method, peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured *in vitro* in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using, *e.g.*, a ^{51}Cr -release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.
- 3) Demonstration of recall T cell responses from patients who have been effectively vaccinated or who have a tumor; (*see, e.g.,* Rehmann, B. *et al.*, *J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997; Tsang *et al.*, *J. Natl. Cancer Inst.* 87:982-990, 1995; Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996). In applying this strategy, recall responses are detected by culturing PBL from patients with cancer who have generated an immune response "naturally", or from patients who were vaccinated with tumor antigen vaccines. PBL from subjects are cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" T cells. At the end of the culture period, T cell activity is detected using assays for T cell activity including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation, or lymphokine release.

The following describes the peptide epitopes and corresponding nucleic acids of the invention.

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IV.C. Binding Affinity of Peptide Epitopes for HLA Molecules

As indicated herein, the large degree of HLA polymorphism is an important factor to be taken into account with the epitope-based approach to vaccine development. To address this factor, epitope selection encompassing identification of peptides capable of binding at high or intermediate affinity to multiple HLA molecules is preferably utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele-specific HLA molecules.

CTL-inducing peptides of interest for vaccine compositions preferably include those that have an IC_{50} or binding affinity value for class I HLA molecules of 500 nM or better (*i.e.*, the value is ≤ 500 nM). HTL-inducing peptides preferably include those that have an IC_{50} or binding affinity value for class II HLA molecules of 1000 nM or better, (*i.e.*, the value is $\leq 1,000$ nM). For example, peptide binding is assessed by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected peptides are tested on other members of the supertype family. In preferred embodiments, peptides that exhibit cross-reactive binding are then used in cellular screening analyses or vaccines.

As disclosed herein, higher HLA binding affinity is correlated with greater immunogenicity. Greater immunogenicity can be manifested in several different ways. Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any particular response, as well as to the extent of a population in which a response is elicited. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. Moreover, higher binding affinity peptides lead to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high or intermediate affinity binding peptide is used. Thus, in preferred embodiments of the invention, high or intermediate affinity binding epitopes are particularly useful.

The relationship between binding affinity for HLA class I molecules and immunogenicity of discrete peptide epitopes on bound antigens has been determined for the first time in the art by the present inventors. The correlation between binding affinity and immunogenicity was analyzed in two different experimental approaches (*see, e.g.*, Sette, *et al.*, *J. Immunol.* 153:5586-5592, 1994). In the first approach, the immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold

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An affinity threshold associated with immunogenicity in the context of HLA class II DR molecules has also been delineated (*see, e.g., Southwood et al. J. Immunology* 160:3363-3373,1998, and co-pending U.S.S.N. 09/009,953 filed 1/21/98). In order to define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element (*i.e., the HLA molecule that binds the motif*) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, *i.e.* binding affinity values of 100 nM or less. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinity values in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC₅₀ of 1000 nM or greater. Thus, 1000 nM can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

In the case of tumor-associated antigens, many CTL peptide epitopes that have been shown to induce CTL that lyse peptide-pulsed target cells and tumor cell targets endogenously expressing the epitope exhibit binding affinity or IC₅₀ values of 200 nM or less. In a study that evaluated the association of binding affinity and immunogenicity of such TAA epitopes, 100% (10/10) of the high binders, *i.e.*, peptide epitopes binding at an affinity of 50 nM or less, were immunogenic and 80% (8/10) of them elicited CTLs that specifically recognized tumor cells. In the 51 to 200 nM range, very similar figures were obtained. CTL inductions positive for peptide and tumor cells were noted for 86% (6/7) and 71% (5/7) of the peptides, respectively. In the 201-500 nM range, most peptides (4/5 wildtype) were positive for induction of CTL recognizing wildtype peptide, but tumor recognition was not detected.

The binding affinity of peptides for HLA molecules can be determined as described in Example 1, below.

IV.D. Peptide Epitope Binding Motifs and Supermotifs

Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues required for allele-specific binding to HLA molecules have been identified. The presence of these residues correlates with binding affinity for HLA molecules. The identification of motifs and/or supermotifs that correlate with high and intermediate affinity binding is an important issue with respect to the identification of immunogenic peptide epitopes for the inclusion in a vaccine. Kast *et al.* (*J. Immunol.* 152:3904-3912, 1994) have shown that motif-bearing peptides account for 90% of the epitopes that bind to allele-specific HLA class I molecules. In this study all possible peptides of 9 amino acids in length and overlapping by eight amino acids (240 peptides), which cover the entire sequence of the E6 and E7 proteins of human papillomavirus type 16, were evaluated for binding to five allele-specific HLA molecules that are expressed at high frequency among different ethnic groups. This unbiased set of peptides allowed an evaluation of the predictive value of HLA class I motifs. From the set of 240 peptides, 22 peptides were identified that bound to an allele-specific HLA molecule with high or intermediate affinity. Of these 22 peptides, 20 (*i.e.* 91%) were motif-bearing. Thus, this study demonstrates the value of motifs for the identification of peptide epitopes for inclusion in a vaccine: application of motif-based identification techniques will identify about 90% of the potential epitopes in a target antigen protein sequence.

Such peptide epitopes are identified in the Tables described below.

Peptides of the present invention may also comprise epitopes that bind to MHC class II DR molecules. A greater degree of heterogeneity in both size and binding frame position of the motif, relative to the N and C termini of the peptide, exists for class II peptide ligands. This increased heterogeneity of HLA class II peptide ligands is due to the structure of the binding groove of the HLA class II molecule which, unlike its class I counterpart, is open at both ends. Crystallographic analysis of HLA class II DRB*0101-peptide complexes showed that the major energy of binding is contributed by peptide residues complexed with complementary pockets on the DRB*0101 molecules. An important anchor residue engages the deepest hydrophobic pocket (*see, e.g.*, Madden, D.R. *Ann. Rev. Immunol.* 13:587, 1995) and is referred to as position 1 (P1). P1 may

represent the N-terminal residue of a class II binding peptide epitope, but more typically is flanked towards the N-terminus by one or more residues. Other studies have also pointed to an important role for the peptide residue in the 6th position towards the C-terminus, relative to P1, for binding to various DR molecules.

5 In the past few years evidence has accumulated to demonstrate that a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets. Thus, peptides of the present invention are identified by any one of several HLA-specific amino acid motifs (*see, e.g.*,
10 Tables I-III), or if the presence of the motif corresponds to the ability to bind several allele-specific HLA molecules, a supermotif. The HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA “supertype.”

The peptide motifs and supermotifs described below, and summarized in Tables I-
15 III, provide guidance for the identification and use of peptide epitopes in accordance with the invention.

Examples of peptide epitopes bearing a respective supermotif or motif are included in Tables as designated in the description of each motif or supermotif below. The Tables include a binding affinity ratio listing for some of the peptide epitopes. The
20 ratio may be converted to IC₅₀ by using the following formula: IC₅₀ of the standard peptide/ratio = IC₅₀ of the test peptide (*i.e.*, the peptide epitope). The IC₅₀ values of standard peptides used to determine binding affinities for Class I peptides are shown in Table IV. The IC₅₀ values of standard peptides used to determine binding affinities for Class II peptides are shown in Table V. The peptides used as standards for the binding
25 assays described herein are examples of standards; alternative standard peptides can also be used when performing binding studies.

To obtain the peptide epitope sequences listed in each Table, protein sequence data for p53 were evaluated for the presence of the designated supermotif or motif. The
30 “pos” (position) column in the Tables designates the amino acid position in the p53 protein that corresponds to the first amino acid residue of the putative epitope. The “number of amino acids” indicates the number of residues in the epitope sequence.

HLA Class I Motifs Indicative of CTL Inducing Peptide Epitopes:

The primary anchor residues of the HLA class I peptide epitope supermotifs and motifs delineated below are summarized in Table I. The HLA class I motifs set out in Table I(a) are those most particularly relevant to the invention claimed here. Primary and secondary anchor positions are summarized in Table II. Allele-specific HLA molecules that comprise HLA class I supertype families are listed in Table VI. In some cases, peptide epitopes may be listed in both a motif and a supermotif Table. The relationship of a particular motif and respective supermotif is indicated in the description of the individual motifs.

IV.D.1. HLA-A1 supermotif

The HLA-A1 supermotif is characterized by the presence in peptide ligands of a small (T or S) or hydrophobic (L, I, V, or M) primary anchor residue in position 2, and an aromatic (Y, F, or W) primary anchor residue at the C-terminal position of the epitope.

The corresponding family of HLA molecules that bind to the A1 supermotif (*i.e.*, the HLA-A1 supertype) is comprised of at least: A*0101, A*2601, A*2602, A*2501, and A*3201 (*see, e.g.*, DiBrino, M. *et al.*, *J. Immunol.* 151:5930, 1993; DiBrino, M. *et al.*, *J. Immunol.* 152:620, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997). Other allele-specific HLA molecules predicted to be members of the A1 superfamily are shown in Table VI. Peptides binding to each of the individual HLA proteins can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A1 supermotif are set forth on the attached Table VII.

IV.D.2. HLA-A2 supermotif

Primary anchor specificities for allele-specific HLA-A2.1 molecules (*see, e.g.*, Falk *et al.*, *Nature* 351:290-296, 1991; Hunt *et al.*, *Science* 255:1261-1263, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992; Ruppert *et al.*, *Cell* 74:929-937, 1993) and cross-reactive binding among HLA-A2 and -A28 molecules have been described. (*See, e.g.*, Fruci *et al.*, *Human Immunol.* 38:187-192, 1993; Tanigaki *et al.*, *Human Immunol.* 39:155-162, 1994; Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994 for reviews of relevant data.) These primary anchor residues define the HLA-A2 supermotif; which presence in peptide ligands corresponds

to the ability to bind several different HLA-A2 and -A28 molecules. The HLA-A2 supermotif comprises peptide ligands with L, I, V, M, A, T, or Q as a primary anchor residue at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope.

- 5 The corresponding family of HLA molecules (*i.e.*, the HLA-A2 supertype that binds these peptides) is comprised of at least: A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, and A*6901. Other allele-specific HLA molecules predicted to be members of the A2 superfamily are shown in Table VI. As explained in detail below, binding to each of the individual allele-specific
- 10 HLA molecules can be modulated by substitutions at the primary anchor and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

- Representative peptide epitopes that comprise an A2 supermotif are set forth on the attached Table VIII. The motifs comprising the primary anchor residues V, A, T, or
- 15 Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.3. HLA-A3 supermotif

- The HLA-A3 supermotif is characterized by the presence in peptide ligands of A,
- 20 L, I, V, M, S, or, T as a primary anchor at position 2, and a positively charged residue, R or K, at the C-terminal position of the epitope, *e.g.*, in position 9 of 9-mers (*see, e.g.*, Sidney *et al.*, *Hum. Immunol.* 45:79, 1996). Exemplary members of the corresponding family of HLA molecules (the HLA-A3 supertype) that bind the A3 supermotif include at least: A*0301, A*1101, A*3101, A*3301, and A*6801. Other allele-specific HLA
- 25 molecules predicted to be members of the A3 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions of amino acids at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

- 30 Representative peptide epitopes that comprise the A3 supermotif are set forth on the attached Table IX.

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IV.D.4. HLA-A24 supermotif

The HLA-A24 supermotif is characterized by the presence in peptide ligands of an aromatic (F, W, or Y) or hydrophobic aliphatic (L, I, V, M, or T) residue as a primary anchor in position 2, and Y, F, W, L, I, or M as primary anchor at the C-terminal position of the epitope (*see, e.g., Sette and Sidney, Immunogenetics, in press, 1999*). The corresponding family of HLA molecules that bind to the A24 supermotif (*i.e., the A24 supertype*) includes at least: A*2402, A*3001, and A*2301. Other allele-specific HLA molecules predicted to be members of the A24 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A24 supermotif are set forth on the attached Table X.

15 IV.D.5. HLA-B7 supermotif

The HLA-B7 supermotif is characterized by peptides bearing proline in position 2 as a primary anchor, and a hydrophobic or aliphatic amino acid (L, I, V, M, A, F, W, or Y) as the primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind the B7 supermotif (*i.e., the HLA-B7 supertype*) is comprised of at least twenty six HLA-B proteins comprising at least: B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, and B*7801 (*see, e.g., Sidney, et al., J. Immunol. 154:247, 1995; Barber, et al., Curr. Biol. 5:179, 1995; Hill, et al., Nature 20 360:434, 1992; Rammensee, et al., Immunogenetics 41:178, 1995 for reviews of relevant data*). Other allele-specific HLA molecules predicted to be members of the B7 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B7 supermotif are set forth on the attached Table XI.

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IV.D.6. HLA-B27 supermotif

The HLA-B27 supermotif is characterized by the presence in peptide ligands of a positively charged (R, H, or K) residue as a primary anchor at position 2, and a hydrophobic (F, Y, L, W, M, I, A, or V) residue as a primary anchor at the C-terminal position of the epitope (*see, e.g.,* Sidney and Sette, *Immunogenetics*, in press, 1999). Exemplary members of the corresponding family of HLA molecules that bind to the B27 supermotif (*i.e.,* the B27 supertype) include at least B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, and B*7301. Other allele-specific HLA molecules predicted to be members of the B27 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B27 supermotif are set forth on the attached Table XII.

IV.D.7. HLA-B44 supermotif

The HLA-B44 supermotif is characterized by the presence in peptide ligands of negatively charged (D or E) residues as a primary anchor in position 2, and hydrophobic residues (F, W, Y, L, I, M, V, or A) as a primary anchor at the C-terminal position of the epitope (*see, e.g.,* Sidney et al., *Immunol. Today* 17:261, 1996). Exemplary members of the corresponding family of HLA molecules that bind to the B44 supermotif (*i.e.,* the B44 supertype) include at least: B*1801, B*1802, B*3701, B*4001, B*4002, B*4006, B*4402, B*4403, and B*4404. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the supermotif.

IV.D.8. HLA-B58 supermotif

The HLA-B58 supermotif is characterized by the presence in peptide ligands of a small aliphatic residue (A, S, or T) as a primary anchor residue at position 2, and an aromatic or hydrophobic residue (F, W, Y, L, I, V, M, or A) as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.,* Sidney and Sette, *Immunogenetics*, in press, 1999 for reviews of relevant data). Exemplary members of the corresponding family of HLA molecules that bind to the B58 supermotif (*i.e.,* the B58 supertype) include at least: B*1516, B*1517, B*5701, B*5702, and B*5801. Other allele-specific

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HLA molecules predicted to be members of the B58 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

- 5 Representative peptide epitopes that comprise the B58 supermotif are set forth on the attached Table XIII.

IV.D.9. HLA-B62 supermotif

- 10 The HLA-B62 supermotif is characterized by the presence in peptide ligands of the polar aliphatic residue Q or a hydrophobic aliphatic residue (L, V, M, I, or P) as a primary anchor in position 2, and a hydrophobic residue (F, W, Y, M, I, V, L, or A) as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics*, in press, 1999). Exemplary members of the corresponding family of HLA molecules that bind to the B62 supermotif (*i.e.*, the B62 supertype) include at least:
- 15 B*1501, B*1502, B*1513, and B5201. Other allele-specific HLA molecules predicted to be members of the B62 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

- 20 Representative peptide epitopes that comprise the B62 supermotif are set forth on the attached Table XIV.

IV.D.10. HLA-A1 motif

- 25 The HLA-A1 motif is characterized by the presence in peptide ligands of T, S, or M as a primary anchor residue at position 2 and the presence of Y as a primary anchor residue at the C-terminal position of the epitope. An alternative allele-specific A1 motif is characterized by a primary anchor residue at position 3 rather than position 2. This motif is characterized by the presence of D, E, A, or S as a primary anchor residue in position 3, and a Y as a primary anchor residue at the C-terminal position of the epitope
- 30 (*see, e.g.*, DiBrino *et al.*, *J. Immunol.*, 152:620, 1994; Kondo *et al.*, *Immunogenetics* 45:249, 1997; and Kubo *et al.*, *J. Immunol.* 152:3913, 1994 for reviews of relevant data). Peptide binding to HLA-A1 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

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Representative peptide epitopes that comprise either A1 motif are set forth on the attached Table XV. Those epitopes comprising T, S, or M at position 2 and Y at the C-terminal position are also included in the listing of HLA-A1 supermotif-bearing peptide epitopes listed in Table VII, as these residues are a subset of the A1 supermotif primary anchors.

IV.D.11. HLA-A*0201 motif

An HLA-A2*0201 motif was determined to be characterized by the presence in peptide ligands of L or M as a primary anchor residue in position 2, and L or V as a primary anchor residue at the C-terminal position of a 9-residue peptide (*see, e.g., Falk et al., Nature* 351:290-296, 1991) and was further found to comprise an I at position 2 and I or A at the C-terminal position of a nine amino acid peptide (*see, e.g., Hunt et al., Science* 255:1261-1263, March 6, 1992; Parker *et al., J. Immunol.* 149:3580-3587, 1992). The A*0201 allele-specific motif has also been defined by the present inventors to additionally comprise V, A, T, or Q as a primary anchor residue at position 2, and M or T as a primary anchor residue at the C-terminal position of the epitope (*see, e.g., Kast et al., J. Immunol.* 152:3904-3912, 1994). Thus, the HLA-A*0201 motif comprises peptide ligands with L, I, V, M, A, T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope. The preferred and tolerated residues that characterize the primary anchor positions of the HLA-A*0201 motif are identical to the residues describing the A2 supermotif. (For reviews of relevant data, *see, e.g., del Guercio et al., J. Immunol.* 154:685-693, 1995; Ruppert *et al., Cell* 74:929-937, 1993; Sidney *et al., Immunol. Today* 17:261-266, 1996; Sette and Sidney, *Curr. Opin. in Immunol.* 10:478-482, 1998). Secondary anchor residues that characterize the A*0201 motif have additionally been defined (*see, e.g., Ruppert et al., Cell* 74:929-937, 1993). These are shown in Table II. Peptide binding to HLA-A*0201 molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise an A*0201 motif are set forth on the attached Table VIII. The A*0201 motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.12. HLA-A3 motif

The HLA-A3 motif is characterized by the presence in peptide ligands of L, M, V, I, S, A, T, F, C, G, or D as a primary anchor residue at position 2, and the presence of K, sY, R, H, F, or A as a primary anchor residue at the C-terminal position of the epitope (see, e.g., DiBrino *et al.*, *Proc. Natl. Acad. Sci USA* 90:1508, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A3 motif are set forth on the attached Table XVI. Those peptide epitopes that also comprise the A3 supermotif are also listed in Table IX. The A3 supermotif primary anchor residues comprise a subset of the A3- and A11-allele specific motif primary anchor residues.

IV.D.13. HLA-A11 motif

The HLA-A11 motif is characterized by the presence in peptide ligands of V, T, M, L, I, S, A, G, N, C, D, or F as a primary anchor residue in position 2, and K, R, Y, or H as a primary anchor residue at the C-terminal position of the epitope (see, e.g., Zhang *et al.*, *Proc. Natl. Acad. Sci USA* 90:2217-2221, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A11 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A11 motif are set forth on the attached Table XVII; peptide epitopes comprising the A3 allele-specific motif are also present in this Table because of the extensive overlap between the A3 and A11 motif primary anchor specificities. Further, those peptide epitopes that comprise the A3 supermotif are also listed in Table IX.

IV.D.14. HLA-A24 motif

The HLA-A24 motif is characterized by the presence in peptide ligands of Y, F, W, or M as a primary anchor residue in position 2, and F, L, I, or W as a primary anchor residue at the C-terminal position of the epitope (see, e.g., Kondo *et al.*, *J. Immunol.* 155:4307-4312, 1995; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A24 molecules can be modulated by substitutions at primary and/or

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secondary anchor positions; preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A24 motif are set forth on the attached Table XVIII. These epitopes are also listed in Table X, which sets forth HLA-A24-supermotif-bearing peptide epitopes, as the primary anchor residues characterizing the A24 allele-specific motif comprise a subset of the A24 supermotif primary anchor residues.

Motifs Indicative of Class II HTL Inducing Peptide Epitopes

The primary and secondary anchor residues of the HLA class II peptide epitope supermotifs and motifs delineated below are summarized in Table III.

IV.D.15. HLA DR-1-4-7 supermotif

Motifs have also been identified for peptides that bind to three common HLA class II allele-specific HLA molecules: HLA DRB1*0401, DRB1*0101, and DRB1*0701 (*see, e.g.,* the review by Southwood *et al. J. Immunology* 160:3363-3373,1998).

Collectively, the common residues from these motifs delineate the HLA DR-1-4-7 supermotif. Peptides that bind to these DR molecules carry a supermotif characterized by a large aromatic or hydrophobic residue (Y, F, W, L, I, V, or M) as a primary anchor residue in position 1, and a small, non-charged residue (S, T, C, A, P, V, I, L, or M) as a primary anchor residue in position 6 of a 9-mer core region. Allele-specific secondary effects and secondary anchors for each of these HLA types have also been identified (Southwood *et al., supra*). These are set forth in Table III. Peptide binding to HLA-DRB1*0401, DRB1*0101, and/or DRB1*0701 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Potential epitope 9-mer core regions comprising the DR-1-4-7 supermotif, wherein position 1 of the supermotif is at position 1 of the nine-residue core, are set forth in Table XIX. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise the nine residue core, are also shown in the Table along with cross-reactive binding data for the exemplary 15-residue supermotif-bearing peptides.

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IV.D.16. HLA DR3 motifs

Two alternative motifs (*i.e.*, submotifs) characterize peptide epitopes that bind to HLA-DR3 molecules (*see, e.g.*, Geluk *et al.*, *J. Immunol.* 152:5742, 1994). In the first motif (submotif DR3a) a large, hydrophobic residue (L, I, V, M, F, or Y) is present in anchor position 1 of a 9-mer core, and D is present as an anchor at position 4, towards the carboxyl terminus of the epitope. As in other class II motifs, core position 1 may or may not occupy the peptide N-terminal position.

The alternative DR3 submotif provides for lack of the large, hydrophobic residue at anchor position 1, and/or lack of the negatively charged or amide-like anchor residue at position 4, by the presence of a positive charge at position 6 towards the carboxyl terminus of the epitope. Thus, for the alternative allele-specific DR3 motif (submotif DR3b): L, I, V, M, F, Y, A, or Y is present at anchor position 1; D, N, Q, E, S, or T is present at anchor position 4; and K, R, or H is present at anchor position 6. Peptide binding to HLA-DR3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Potential peptide epitope 9-mer core regions corresponding to a nine residue sequence comprising the DR3a submotif (wherein position 1 of the motif is at position 1 of the nine residue core) are set forth in Table XXa. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise the nine residue core, are also shown in Table XXa along with binding data for the exemplary DR3 submotif a-bearing peptides.

Potential peptide epitope 9-mer core regions comprising the DR3b submotif and respective exemplary 15-mer peptides comprising the DR3 submotif-b epitope are set forth in Table XXb along with binding data for the exemplary DR3 submotif b-bearing peptides.

Each of the HLA class I or class II peptide epitopes set out in the Tables herein are deemed singly to be an inventive aspect of this application. Further, it is also an inventive aspect of this application that each peptide epitope may be used in combination with any other peptide epitope.

IV.E. Enhancing Population Coverage of the Vaccine

Vaccines that have broad population coverage are preferred because they are more commercially viable and generally applicable to the most people. Broad population coverage can be obtained using the peptides of the invention (and nucleic acid

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compositions that encode such peptides) through selecting peptide epitopes that bind to HLA alleles which, when considered in total, are present in most of the population. Table XXI lists the overall frequencies of the HLA class I supertypes in various ethnicities (Table XXIa) and the combined population coverage achieved by the A2-, A3-, and B7-
 5 supertypes (Table XXIb). The A2-, A3-, and B7 supertypes are each present on the average of over 40% in each of these five major ethnic groups. Coverage in excess of 80% is achieved with a combination of these supermotifs. These results suggest that effective and non-ethnically biased population coverage is achieved upon use of a limited number of cross-reactive peptides. Although the population coverage reached with these
 10 three main peptide specificities is high, coverage can be expanded to reach 95% population coverage and above, and more easily achieve truly multispecific responses upon use of additional supermotif or allele-specific motif bearing peptides.

The B44-, A1-, and A24-supertypes are each present, on average, in a range from 25% to 40% in these major ethnic populations (Table XXIa). While less prevalent
 15 overall, the B27-, B58-, and B62 supertypes are each present with a frequency >25% in at least one major ethnic group (Table XXIa). Table XXIb summarizes the estimated prevalence of combinations of HLA supertypes that have been identified in five major ethnic groups. The incremental coverage obtained by the inclusion of A1-, A24-, and B44-supertypes to the A2, A3, and B7 coverage and coverage obtained with all of the
 20 supertypes described herein, is shown.

The data presented herein, together with the previous definition of the A2-, A3-, and B7-supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. By including epitopes from the six most frequent supertypes, an average population coverage of 99% is obtained
 25 for five major ethnic groups.

IV.F. Immune Response-Stimulating Peptide Analogs

In general, CTL and HTL responses are not directed against all possible epitopes. Rather, they are restricted to a few "immunodominant" determinants (Zinkernagel, *et al.*,
 30 *Adv. Immunol.* 27:5159, 1979; Bennink, *et al.*, *J. Exp. Med.* 168:1935-1939, 1988; Rawle, *et al.*, *J. Immunol.* 146:3977-3984, 1991). It has been recognized that immunodominance (Benacerraf, *et al.*, *Science* 175:273-279, 1972) could be explained by either the ability of a given epitope to selectively bind a particular HLA protein (determinant selection theory) (Vitiello, *et al.*, *J. Immunol.* 131:1635, 1983); Rosenthal, *et al.*, *Nature* 267:156-

158, 1977), or to be selectively recognized by the existing TCR (T cell receptor) specificities (repertoire theory) (Klein, J., IMMUNOLOGY, THE SCIENCE OF SELF/NONSELF DISCRIMINATION, John Wiley & Sons, New York, pp. 270-310, 1982). It has been demonstrated that additional factors, mostly linked to processing events, can also play a key role in dictating, beyond strict immunogenicity, which of the many potential determinants will be presented as immunodominant (Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993).

Because tissue specific and developmental TAAs are expressed on normal tissue at least at some point in time or location within the body, it may be expected that T cells to them, particularly dominant epitopes, are eliminated during immunological surveillance and that tolerance is induced. However, CTL responses to tumor epitopes in both normal donors and cancer patient has been detected, which may indicate that tolerance is incomplete (*see, e.g.*, Kawashima *et al.*, *Hum. Immunol.* 59:1, 1998; Tsang, *J. Natl. Cancer Inst.* 87:82-90, 1995; Rongcun *et al.*, *J. Immunol.* 163:1037, 1999). Thus, immune tolerance does not completely eliminate or inactivate CTL precursors capable of recognizing high affinity HLA class I binding peptides.

An additional strategy to overcome tolerance is to use analog peptides. Without intending to be bound by theory, it is believed that because T cells to dominant epitopes may have been clonally deleted, selecting subdominant epitopes may allow existing T cells to be recruited, which will then lead to a therapeutic or prophylactic response. However, the binding of HLA molecules to subdominant epitopes is often less vigorous than to dominant ones. Accordingly, there is a need to be able to modulate the binding affinity of particular immunogenic epitopes for one or more HLA molecules, and thereby to modulate the immune response elicited by the peptide, for example to prepare analog peptides which elicit a more vigorous response.

Although peptides with suitable cross-reactivity among all alleles of a superfamily are identified by the screening procedures described above, cross-reactivity is not always as complete as possible, and in certain cases procedures to increase cross-reactivity of peptides can be useful; moreover, such procedures can also be used to modify other properties of the peptides such as binding affinity or peptide stability. Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.*, analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-

reactivity patterns, can be produced in accordance with the teachings herein. The present concepts related to analog generation are set forth in greater detail in co-pending U.S.S.N. 09/226,775 filed 1/6/99.

In brief, the strategy employed utilizes the motifs or supermotifs which correlate
 5 with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, and in many cases secondary anchors. Analog peptides can be created by substituting amino acid residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and
 10 motifs that have been defined for HLA class I and class II binding peptides are shown in Tables II and III, respectively.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind the respective motif or supermotif (Tables II and
 15 III). Accordingly, removal of such residues that are detrimental to binding can be performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of peptides used in the analysis, the incidence of cross-reactivity increased from 22% to 37% (*see, e.g.,* Sidney, J. *et al., Hu. Immunol.* 45:79, 1996). Thus, one
 20 strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, "preferred" residues associated with
 25 high affinity binding to an allele-specific HLA molecule or to multiple HLA molecules within a superfamily are inserted.

To ensure that an analog peptide, when used as a vaccine, actually elicits a CTL response to the native epitope *in vivo* (or, in the case of class II epitopes, elicits helper T cells that cross-react with the wild type peptides), the analog peptide may be used to
 30 immunize T cells *in vitro* from individuals of the appropriate HLA allele. Thereafter, the immunized cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. It will be desirable to use as antigen presenting cells, cells that have been either infected, or transfected with the appropriate genes, or, in the case of class II

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epitopes only, cells that have been pulsed with whole protein antigens, to establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention is to create analogs of weak binding peptides, to thereby ensure adequate numbers of cross-reactive cellular binders. Class I
 5 binding peptides exhibiting binding affinities of 500-5000 nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be "fixed" by substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the
 10 substitution of residues that have an adverse impact on peptide stability or solubility in, *e.g.*, a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine can be substituted out in favor of α -amino butyric acid ("B" in the single letter abbreviations for peptide sequences listed herein). Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently
 15 alter the peptide structurally so as to reduce binding capacity. Substituting α -amino butyric acid for cysteine not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (*see, e.g.*, the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

20 Representative analog peptides are set forth in Table XXII. The Table indicates the length and sequence of the analog peptide as well as the motif or supermotif, if appropriate. The information in the "Fixed Nomenclature" column indicates the residues substituted at the indicated position numbers for the respective analog.

25 IV.G. Computer Screening of Protein Sequences from Disease-Related Antigens for Supermotif- or Motif-Bearing Peptides

In order to identify supermotif- or motif-bearing epitopes in a target antigen, a native protein sequence, *e.g.*, a tumor-associated antigen, or sequences from an infectious organism, or a donor tissue for transplantation, is screened using a means for computing,
 30 such as an intellectual calculation or a computer, to determine the presence of a supermotif or motif within the sequence. The information obtained from the analysis of native peptide can be used directly to evaluate the status of the native peptide or may be utilized subsequently to generate the peptide epitope.

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Computer programs that allow the rapid screening of protein sequences for the occurrence of the subject supermotifs or motifs are encompassed by the present invention; as are programs that permit the generation of analog peptides. These programs are implemented to analyze any identified amino acid sequence or operate on an unknown sequence and simultaneously determine the sequence and identify motif-bearing epitopes thereof; analogs can be simultaneously determined as well. Generally, the identified sequences will be from a pathogenic organism or a tumor-associated peptide. For example, the target TAA molecules include, without limitation, CEA, MAGE, p53 and HER2/neu.

It is important that the selection criteria utilized for prediction of peptide binding are as accurate as possible, to correlate most efficiently with actual binding. Prediction of peptides that bind, for example, to HLA-A*0201, on the basis of the presence of the appropriate primary anchors, is positive at about a 30% rate (*see, e.g.,* Ruppert, J. *et al. Cell* 74:929, 1993). However, by extensively analyzing peptide-HLA binding data disclosed herein, data in related patent applications, and data in the art, the present inventors have developed a number of allele-specific polynomial algorithms that dramatically increase the predictive value over identification on the basis of the presence of primary anchor residues alone. These algorithms take into account not only the presence or absence of primary anchors, but also consider the positive or deleterious presence of secondary anchor residues (to account for the impact of different amino acids at different positions). The algorithms are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA interactions can be approximated as a linear polynomial function of the type:

$$\Delta G = a_{1j} \times a_{2i} \times a_{3j} \dots \times a_{ni}$$

where a_{ji} is a coefficient that represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. An important assumption of this method is that the effects at each position are essentially independent of each other. This assumption is justified by studies that demonstrated that peptides are bound to HLA molecules and recognized by T cells in essentially an extended conformation. Derivation of specific algorithm coefficients has been described, for example, in Gulukota, K. *et al., J. Mol. Biol.* 267:1258, 1997.

Additional methods to identify preferred peptide sequences, which also make use of specific motifs, include the use of neural networks and molecular modeling programs

(see, e.g., Milik *et al.*, *Nature Biotechnology* 16:753, 1998; Altuvia *et al.*, *Hum. Immunol.* 58:1, 1997; Altuvia *et al.*, *J. Mol. Biol.* 249:244, 1995; Buus, S. *Curr. Opin. Immunol.* 11:209-213, 1999; Brusic, V. *et al.*, *Bioinformatics* 14:121-130, 1998; Parker *et al.*, *J. Immunol.* 152:163, 1993; Meister *et al.*, *Vaccine* 13:581, 1995; Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994; Sturniolo *et al.*, *Nature Biotechnol.* 17:555 1999).

For example, it has been shown that in sets of A*0201 motif-bearing peptides containing at least one preferred secondary anchor residue while avoiding the presence of any deleterious secondary anchor residues, 69% of the peptides will bind A*0201 with an IC₅₀ less than 500 nM (Ruppert, J. *et al. Cell* 74:929, 1993). These algorithms are also flexible in that cut-off scores may be adjusted to select sets of peptides with greater or lower predicted binding properties, as desired.

In utilizing computer screening to identify peptide epitopes, a protein sequence or translated sequence may be analyzed using software developed to search for motifs, for example the "FINDPATTERNS" program (Devereux, *et al. Nucl. Acids Res.* 12:387-395, 1984) or MotifSearch 1.4 software program (D. Brown, San Diego, CA) to identify potential peptide sequences containing appropriate HLA binding motifs. The identified peptides can be scored using customized polynomial algorithms to predict their capacity to bind specific HLA class I or class II alleles. As appreciated by one of ordinary skill in the art, a large array of computer programming software and hardware options are available in the relevant art which can be employed to implement the motifs of the invention in order to evaluate (e.g., without limitation, to identify epitopes, identify epitope concentration per peptide length, or to generate analogs) known or unknown peptide sequences.

In accordance with the procedures described above, p53 peptide epitopes and analogs thereof that are able to bind HLA supertype groups or allele-specific HLA molecules have been identified (Tables VII-XX; Table XXII).

IV.H. Preparation of Peptide Epitopes

Peptides in accordance with the invention can be prepared synthetically, by recombinant DNA technology or chemical synthesis, or from natural sources such as native tumors or pathogenic organisms. Peptide epitopes may be synthesized individually or as polyepitopic peptides. Although the peptide will preferably be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides may be synthetically conjugated to native fragments or particles.

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The peptides in accordance with the invention can be a variety of lengths, and either in their neutral (uncharged) forms or in forms which are salts. The peptides in accordance with the invention are either free of modifications such as glycosylation, side chain oxidation, or phosphorylation; or they contain these modifications, subject to the condition that modifications do not destroy the biological activity of the peptides as described herein.

Desirably, the peptide epitope will be as small as possible while still maintaining substantially all of the immunologic activity of the native protein. When possible, it may be desirable to optimize HLA class I binding peptide epitopes of the invention to a length of about 8 to about 13 amino acid residues, preferably 9 to 10. HLA class II binding peptide epitopes may be optimized to a length of about 6 to about 30 amino acids in length, preferably to between about 13 and about 20 residues. Preferably, the peptide epitopes are commensurate in size with endogenously processed pathogen-derived peptides or tumor cell peptides that are bound to the relevant HLA molecules.

The identification and preparation of peptides of other lengths can also be carried out using the techniques described herein. Moreover, it is preferred to identify native peptide regions that contain a high concentration of class I and/or class II epitopes. Such a sequence is generally selected on the basis that it contains the greatest number of epitopes per amino acid length. It is to be appreciated that epitopes can be present in a frame-shifted manner, *e.g.* a 10 amino acid long peptide could contain two 9 amino acid long epitopes and one 10 amino acid long epitope; upon intracellular processing, each epitope can be exposed and bound by an HLA molecule upon administration of such a peptide. This larger, preferably multi-epitopic, peptide can be generated synthetically, recombinantly, or via cleavage from the native source.

The peptides of the invention can be prepared in a wide variety of ways. For the preferred relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. (*See, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co., 1984*). Further, individual peptide epitopes can be joined using chemical ligation to produce larger peptides that are still within the bounds of the invention.

Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated

under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Thus, recombinant polypeptides which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

The nucleotide coding sequence for peptide epitopes of the preferred lengths contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, *et al.*, *J. Am. Chem. Soc.* 103:3185 (1981). Peptide analogs can be made simply by substituting the appropriate and desired nucleic acid base(s) for those that encode the native peptide sequence; exemplary nucleic acid substitutions are those that encode an amino acid defined by the motifs/super motifs herein. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast, insect or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

IV.I. Assays to Detect T-Cell Responses

Once HLA binding peptides are identified, they can be tested for the ability to elicit a T-cell response. The preparation and evaluation of motif-bearing peptides are described in PCT publications WO 94/20127 and WO 94/03205. Briefly, peptides comprising epitopes from a particular antigen are synthesized and tested for their ability to bind to the appropriate HLA proteins. These assays may involve evaluating the binding of a peptide of the invention to purified HLA class I molecules in relation to the binding of a radioiodinated reference peptide. Alternatively, cells expressing empty class I molecules (*i.e.* lacking peptide therein) may be evaluated for peptide binding by immunofluorescent staining and flow microfluorimetry. Other assays that may be used to evaluate peptide binding include peptide-dependent class I assembly assays and/or the

inhibition of CTL recognition by peptide competition. Those peptides that bind to the class I molecule, typically with an affinity of 500 nM or less, are further evaluated for their ability to serve as targets for CTLs derived from infected or immunized individuals, as well as for their capacity to induce primary *in vitro* or *in vivo* CTL responses that can
 5 give rise to CTL populations capable of reacting with selected target cells associated with a disease. Corresponding assays are used for evaluation of HLA class II binding peptides. HLA class II motif-bearing peptides that are shown to bind, typically at an affinity of 1000 nM or less, are further evaluated for the ability to stimulate HTL responses.

Conventional assays utilized to detect T cell responses include proliferation
 10 assays, lymphokine secretion assays, direct cytotoxicity assays, and limiting dilution assays. For example, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells. Alternatively, mutant non-human mammalian cell lines that are
 15 deficient in their ability to load class I molecules with internally processed peptides and that have been transfected with the appropriate human class I gene, may be used to test for the capacity of the peptide to induce *in vitro* primary CTL responses.

Peripheral blood mononuclear cells (PBMCs) may be used as the responder cell source of CTL precursors. The appropriate antigen-presenting cells are incubated with
 20 peptide, after which the peptide-loaded antigen-presenting cells are then incubated with the responder cell population under optimized culture conditions. Positive CTL activation can be determined by assaying the culture for the presence of CTLs that kill radio-labeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed forms of the antigen from which the peptide sequence
 25 was derived.

More recently, a method has been devised which allows direct quantification of antigen-specific T cells by staining with Fluorescein-labelled HLA tetrameric complexes (Altman, J. D. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10330, 1993; Altman, J. D. *et al.*, *Science* 274:94, 1996). Other relatively recent technical developments include staining
 30 for intracellular lymphokines, and interferon- γ release assays or ELISPOT assays. Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Lalvani, A. *et al.*, *J. Exp.*

Med. 186:859, 1997; Dunbar, P. R. *et al.*, *Curr. Biol.* 8:413, 1998; Murali-Krishna, K. *et al.*, *Immunity* 8:177, 1998).

HTL activation may also be assessed using such techniques known to those in the art such as T cell proliferation and secretion of lymphokines, *e.g.* IL-2 (*see, e.g.*

5 Alexander *et al.*, *Immunity* 1:751-761, 1994).

Alternatively, immunization of HLA transgenic mice can be used to determine immunogenicity of peptide epitopes. Several transgenic mouse models including mice with human A2.1, A11 (which can additionally be used to analyze HLA-A3 epitopes), and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and 10 A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed. Additional transgenic mouse models with other HLA alleles may be generated as necessary. Mice may be immunized with peptides emulsified in Incomplete Freund's Adjuvant and the resulting T cells tested for their capacity to recognize peptide-pulsed target cells and target cells transfected with appropriate genes. CTL responses 15 may be analyzed using cytotoxicity assays described above. Similarly, HTL responses may be analyzed using such assays as T cell proliferation or secretion of lymphokines.

Exemplary immunogenic peptide epitopes are set out in Table XXIII.

20 **IV.J. Use of Peptide Epitopes as Diagnostic Agents and for Evaluating Immune Responses**

HLA class I and class II binding peptides as described herein can be used, in one embodiment of the invention, as reagents to evaluate an immune response. The immune response to be evaluated may be induced by using as an immunogen any agent that may result in the production of antigen-specific CTLs or HTLs that recognize and bind to the peptide epitope(s) to be employed as the reagent. The peptide reagent need not be used as 25 the immunogen. Assay systems that may be used for such an analysis include relatively recent technical developments such as tetramers, staining for intracellular lymphokines and interferon release assays, or ELISPOT assays.

For example, a peptide of the invention may be used in a tetramer staining assay 30 to assess peripheral blood mononuclear cells for the presence of antigen-specific CTLs following exposure to a tumor cell antigen or an immunogen. The HLA-tetrameric complex is used to directly visualize antigen-specific CTLs (*see, e.g.*, Ogg *et al.*, *Science* 279:2103-2106, 1998; and Altman *et al.*, *Science* 174:94-96, 1996) and determine the frequency of the antigen-specific CTL population in a sample of peripheral blood

mononuclear cells. A tetramer reagent using a peptide of the invention may be generated as follows: A peptide that binds to an HLA molecule is refolded in the presence of the corresponding HLA heavy chain and β_2 -microglobulin to generate a trimolecular complex. The complex is biotinylated at the carboxyl terminal end of the heavy chain at a site that was previously engineered into the protein. Tetramer formation is then induced by the addition of streptavidin. By means of fluorescently labeled streptavidin, the tetramer can be used to stain antigen-specific cells. The cells may then be identified, for example, by flow cytometry. Such an analysis may be used for diagnostic or prognostic purposes.

Peptides of the invention may also be used as reagents to evaluate immune recall responses (*see, e.g., Berton et al., J. Clin. Invest.* 100:503-513, 1997 and Penna *et al., J. Exp. Med.* 174:1565-1570, 1991). For example, patient PBMC samples from individuals with cancer may be analyzed for the presence of antigen-specific CTLs or HTLs using specific peptides. A blood sample containing mononuclear cells may be evaluated by cultivating the PBMCs and stimulating the cells with a peptide of the invention. After an appropriate cultivation period, the expanded cell population may be analyzed, for example, for CTL or for HTL activity.

The peptides may also be used as reagents to evaluate the efficacy of a vaccine. PBMCs obtained from a patient vaccinated with an immunogen may be analyzed using, for example, either of the methods described above. The patient is HLA typed, and peptide epitope reagents that recognize the allele-specific molecules present in that patient are selected for the analysis. The immunogenicity of the vaccine is indicated by the presence of epitope-specific CTLs and/or HTLs in the PBMC sample.

The peptides of the invention may also be used to make antibodies, using techniques well known in the art (*see, e.g. CURRENT PROTOCOLS IN IMMUNOLOGY*, Wiley/Greene, NY; and *Antibodies A Laboratory Manual*, Harlow and Lane, Cold Spring Harbor Laboratory Press, 1989), which may be useful as reagents to diagnose or monitor cancer. Such antibodies include those that recognize a peptide in the context of an HLA molecule, *i.e.*, antibodies that bind to a peptide-MHC complex.

IV.K. Vaccine Compositions

Vaccines that contain an immunogenically effective amount of one or more peptides as described herein are a further embodiment of the invention. Once

- appropriately immunogenic epitopes have been defined, they can be sorted and delivered by various means, herein referred to as "vaccine" compositions. Such vaccine compositions can include, for example, lipopeptides (*e.g.*, Vitiello, A. *et al.*, *J. Clin. Invest.* 95:341, 1995), peptide compositions encapsulated in poly(DL-lactide-co-glycolide) ("PLG") microspheres (*see, e.g.*, Eldridge, *et al.*, *Molec. Immunol.* 28:287-294, 1991; Alonso *et al.*, *Vaccine* 12:299-306, 1994; Jones *et al.*, *Vaccine* 13:675-681, 1995), peptide compositions contained in immune stimulating complexes (ISCOMS) (*see, e.g.*, Takahashi *et al.*, *Nature* 344:873-875, 1990; Hu *et al.*, *Clin Exp Immunol.* 113:235-243, 1998), multiple antigen peptide systems (MAPs) (*see e.g.*, Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.* 85:5409-5413, 1988; Tam, J.P., *J. Immunol. Methods* 196:17-32, 1996), viral delivery vectors (Perkus, M. E. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 379, 1996; Chakrabarti, S. *et al.*, *Nature* 320:535, 1986; Hu, S. L. *et al.*, *Nature* 320:537, 1986; Kieny, M.-P. *et al.*, *AIDS Bio/Technology* 4:790, 1986; Top, F. H. *et al.*, *J. Infect. Dis.* 124:148, 1971; Chanda, P. K. *et al.*, *Virology* 175:535, 1990), particles of viral or synthetic origin (*e.g.*, Kofler, N. *et al.*, *J. Immunol. Methods* 192:25, 1996; Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993; Falo, L. D., Jr. *et al.*, *Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. *et al.*, *Vaccine* 11:293, 1993), liposomes (Reddy, R. *et al.*, *J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or, naked or particle absorbed cDNA (Ulmer, J. B. *et al.*, *Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.

Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the claimed peptide(s). The peptide(s) can be individually linked to its own carrier; alternatively, the peptide(s) can exist as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptide epitopes are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targeted for an immune

response. The composition may be a naturally occurring region of an antigen or may be prepared, *e.g.*, recombinantly or by chemical synthesis.

Furthermore, useful carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (*i.e.*, acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are examples of materials well known in the art. Additionally, as disclosed herein, CTL responses can be primed by conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycerylcysteinylserine (P₃CSS).

As disclosed in greater detail herein, upon immunization with a peptide composition in accordance with the invention, via injection, aerosol, oral, transdermal, transmucosal, intrapleural, intrathecal, or other suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later infection, or at least partially resistant to developing an ongoing chronic infection, or derives at least some therapeutic benefit when the antigen was tumor-associated.

In some instances it may be desirable to combine the class I peptide vaccines of the invention with vaccines which induce or facilitate neutralizing antibody responses to the target antigen of interest, particularly to viral envelope antigens. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I and/or class II epitope in accordance with the invention, along with a PADRE™ (Epimmune, San Diego, CA) molecule (described, for example, in U.S. Patent Number 5,736,142). Furthermore, any of these embodiments can be administered as a nucleic acid mediated modality.

For therapeutic or prophylactic immunization purposes, the peptides of the invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus, for example, as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into a host

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bearing a tumor, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL and/or HTL response. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 5 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, *e.g.* adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.

Antigenic peptides are used to elicit a CTL and/or HTL response *ex vivo*, as well. 10 The resulting CTL or HTL cells, can be used to treat chronic infections, or tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL or HTL responses to a particular antigen (infectious or tumor-associated antigen) are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or 15 HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cell (an infected cell 20 or a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells. Alternatively, dendritic cells are transfected, *e.g.*, with a minigene construct in accordance with the invention, in order to elicit immune responses. Minigenes will be discussed in greater detail in a following section.

Vaccine compositions may also be administered *in vivo* in combination with 25 dendritic cell mobilization whereby loading of dendritic cells occurs *in vivo*.

DNA or RNA encoding one or more of the peptides of the invention can also be administered to a patient. This approach is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of 30 DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (*see, e.g.*, U.S. Patent No. 5,922,687).

Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polypeptidic composition for use in a vaccine, or for selecting

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discrete epitopes to be included in a vaccine and/or to be encoded by nucleic acids such as a minigene. Exemplary epitopes that may be utilized in a vaccine to treat or prevent cancer are set out in Tables XXXVII and XXXVIII. It is preferred that each of the following principles are balanced in order to make the selection. The multiple epitopes to be incorporated in a given vaccine composition may be, but need not be, contiguous in sequence in the native antigen from which the epitopes are derived.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For HLA Class I this includes 3-4 epitopes that come from at least one TAA. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one TAA (*see e.g.*, Rosenberg *et al.*, *Science* 278:1447-1450). Epitopes from one TAA may be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, *e.g.*, in Example 15.

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less.

3.) Sufficient supermotif bearing-peptides, or a sufficient array of allele-specific motif-bearing peptides, are selected to give broad population coverage. For example, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess the breadth, or redundancy of, population coverage.

4.) When selecting epitopes from cancer-related antigens it is often preferred to select analogs because the patient may have developed tolerance to the native epitope.

When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes. Of particular relevance for infectious disease vaccines (but for cancer-related vaccines as well), are epitopes referred to as "nested epitopes."

Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it.

When providing nested epitopes, it is preferable to provide a sequence that has the greatest number of epitopes per provided sequence. Preferably, one avoids providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a

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longer peptide sequence, such as a sequence comprising nested epitopes, it is important to screen the sequence in order to insure that it does not have pathological or other deleterious biological properties.

- 5.) When creating a minigene, as disclosed in greater detail in the following section, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those employed when selecting a peptide comprising nested epitopes. Furthermore, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, *e.g.*, by motif analysis, that only exists because two discrete peptide sequences are encoded directly next to each other. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that non-native epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

IV.K.1. Minigene Vaccines

- A growing body of experimental evidence demonstrates that a number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the peptides of the invention are a particularly useful embodiment of the invention. Epitopes for inclusion in a minigene are preferably selected according to the guidelines set forth in the previous section. A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding a peptide comprising one or multiple epitopes of the invention. The use of multi-epitope minigenes is described below and in, *e.g.*, co-pending application U.S.S.N. 09/311,784; Ishioka *et al.*, *J. Immunol.* 162:3915-3925, 1999; An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J. Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding supermotif- and/or motif-bearing p53 epitopes derived from multiple regions of p53, the PADRE™ universal helper T cell epitope (or multiple HTL epitopes from p53), and an endoplasmic reticulum-translocating signal sequence can

be engineered. A vaccine may also comprise epitopes, in addition to p53 epitopes, that are derived from other TAAs.

The immunogenicity of a multi-epitopic minigene can be tested in transgenic mice to evaluate the magnitude of CTL induction responses against the epitopes tested.

5 Further, the immunogenicity of DNA-encoded epitopes *in vivo* can be correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these experiments can show that the minigene serves to both: 1.) generate a CTL response and 2.) that the induced CTLs recognized cells expressing the encoded epitopes.

10 For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression
15 and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that can be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including
20 synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides
25 (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are
30 preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus

(hCMV) promoter. See, *e.g.*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (*e.g.*, IL-2, IL-12, GM-CSF), cytokine-inducing molecules (*e.g.*, LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins (PADRE™, Epimmune, San Diego, CA). Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (*e.g.* TGF-β) may be beneficial in certain diseases.

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor

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according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

5 Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffered saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for
10 formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids, glycolipids, and fusogenic liposomes can also be used in the formulation (see, *e.g.*, as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987).
15 In addition, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

Target cell sensitization can be used as a functional assay for expression and HLA
20 class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be
25 co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and used as target cells for epitope-specific CTL lines; cytotoxicity, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of HTL epitopes may be evaluated in an analogous manner using assays to assess HTL
30 activity.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (*e.g.*, IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA).

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Twenty-one days after immunization, splenocytes are harvested and restimulated for one week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ⁵¹Cr-labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccine function for *in vivo* induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

IV.K.2. Combinations of CTL Peptides with Helper Peptides

Vaccine compositions comprising the peptides of the present invention, or analogs thereof, which have immunostimulatory activity may be modified to provide desired attributes, such as improved serum half-life, or to enhance immunogenicity.

For instance, the ability of a peptide to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. The use of T helper epitopes in conjunction with CTL epitopes to enhance immunogenicity is illustrated, for example, in the co-pending applications U.S.S.N. 08/820,360, U.S.S.N. 08/197,484, and U.S.S.N. 08/464,234.

Particularly preferred CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, *e.g.*, Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL peptide may be linked to the T helper peptide without a spacer.

The CTL peptide epitope may be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide may be acylated. The HTL peptide epitopes used in the invention can be modified in the same

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manner as CTL peptides. For instance, they may be modified to include D-amino acids or be conjugated to other molecules such as lipids, proteins, sugars and the like.

~~In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting~~

5 amino acid sequences that bind to many, most, or all of the HLA class II molecules. These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences. Examples of amino acid sequences that are promiscuous include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), *Plasmodium falciparum* CS protein at positions 378-398 (DIEKKIAKMEKASSVFNVNS), and
10 Streptococcus 18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs,

~~Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (see, e.g., PCT publication WO 95/07707). These synthetic compounds~~
15 called Pan-DR-binding epitopes (e.g., PADRE™, Epimmune, Inc., San Diego, CA) are designed to most preferably bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVWANTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and "a" is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the
20 response of T helper lymphocytes from most individuals, regardless of their HLA type.

An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

HTL peptide epitopes can also be modified to alter their biological properties. For example, peptides comprising HTL epitopes can contain D-amino acids to increase their
25 resistance to proteases and thus extend their serum half-life. Also, the epitope peptides of the invention can be conjugated to other molecules such as lipids, proteins or sugars, or any other synthetic compounds, to increase their biological activity. Specifically, the T helper peptide can be conjugated to one or more palmitic acid chains at either the amino or carboxyl termini.

30 In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the ε- and α-

amino groups of a lysine residue and then linked, *e.g.*, via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant, *e.g.*, incomplete Freund's
 5 adjuvant. A particularly effective immunogen comprises palmitic acid attached to ϵ - and α - amino groups of Lys, which is attached via linkage, *e.g.*, Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylserine (P₃CSS) can be used to prime virus
 10 specific CTL when covalently attached to an appropriate peptide (*see, e.g.*, Deres, *et al.*, *Nature* 342:561, 1989). Peptides of the invention can be coupled to P₃CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P₃CSS-conjugated epitopes, two such compositions can be combined to more
 15 effectively elicit both humoral and cell-mediated responses to infection.

As noted herein, additional amino acids can be added to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or
 20 aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide, particularly class I peptides. However, it is to be noted that modification at the carboxyl terminus of a CTL epitope may, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH₂ acylation, *e.g.*, by alkanoyl (C₁-C₂₀)
 25 or thioglycolyl acetylation, terminal-carboxyl amidation, *e.g.*, ammonia, methylamine, *etc.* In some instances these modifications may provide sites for linking to a support or other molecule.

IV.L. Administration of Vaccines for Therapeutic or Prophylactic Purposes

30 The peptides of the present invention and pharmaceutical and vaccine compositions of the invention are useful for administration to mammals, particularly humans, to treat and/or prevent cancer. Vaccine compositions containing the peptides of the invention are administered to a cancer patient or to an individual susceptible to, or

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otherwise at risk for, cancer to elicit an immune response against TAAs and thus enhance the patient's own immune response capabilities. In therapeutic applications, peptide and/or nucleic acid compositions are administered to a patient in an amount sufficient to elicit an effective CTL and/or HTL response to the tumor antigen and to cure or at least partially arrest or slow symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the particular composition administered, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

The vaccine compositions of the invention may also be used purely as prophylactic agents. Generally the dosage for an initial prophylactic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg . Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. This is followed by boosting dosages of between about 1.0 μg to about 50,000 μg of peptide administered at defined intervals from about four weeks to six months after the initial administration of vaccine. The immunogenicity of the vaccine may be assessed by measuring the specific activity of CTL and HTL obtained from a sample of the patient's blood.

As noted above, peptides comprising CTL and/or HTL epitopes of the invention induce immune responses when presented by HLA molecules and contacted with a CTL or HTL specific for an epitope comprised by the peptide. The manner in which the peptide is contacted with the CTL or HTL is not critical to the invention. For instance, the peptide can be contacted with the CTL or HTL either *in vivo* or *in vitro*. If the contacting occurs *in vivo*, the peptide itself can be administered to the patient, or other vehicles, *e.g.*, DNA vectors encoding one or more peptides, viral vectors encoding the peptide(s), liposomes and the like, can be used, as described herein.

When the peptide is contacted *in vitro*, the vaccinating agent can comprise a population of cells, *e.g.*, peptide-pulsed dendritic cells, or TAA-specific CTLs, which have been induced by pulsing antigen-presenting cells *in vitro* with the peptide. Such a cell population is subsequently administered to a patient in a therapeutically effective dose.

For pharmaceutical compositions, the immunogenic peptides of the invention, or DNA encoding them, are generally administered to an individual already diagnosed with

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cancer. The peptides or DNA encoding them can be administered individually or as fusions of one or more peptide sequences.

For therapeutic use, administration should generally begin at the first diagnosis of cancer. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. The embodiment of the vaccine composition (i.e., including, but not limited to embodiments such as peptide cocktails, polypeptides, minigenes, or TAA-specific CTLs) delivered to the patient may vary according to the stage of the disease. For example, a vaccine comprising TAA-specific CTLs may be more efficacious in killing tumor cells in patients with advanced disease than alternative embodiments.

The vaccine compositions of the invention may also be used therapeutically in combination with treatments such as surgery. An example is a situation in which a patient has undergone surgery to remove a primary tumor and the vaccine is then used to slow or prevent recurrence and/or metastasis.

Where susceptible individuals, e.g., individuals who may be diagnosed as being genetically pre-disposed to developing a particular type of tumor, are identified prior to diagnosis of cancer, the composition can be targeted to them, thus minimizing the need for administration to a larger population.

The dosage for an initial therapeutic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1,000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg . Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. Boosting dosages of between about 1.0 μg to about 50,000 μg of peptide pursuant to a boosting regimen over weeks to months may be administered depending upon the patient's response and condition as determined by measuring the specific activity of CTL and HTL obtained from the patient's blood. The peptides and compositions of the present invention may be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, as a result of the minimal amounts of extraneous substances and the relative nontoxic nature of the peptides in preferred compositions of the invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions relative to these stated dosage amounts.

Thus, for treatment of cancer, a representative dose is in the range disclosed above, namely where the lower value is about 1, 5, 50, 500, or 1,000 μg and the higher

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value is about 10,000; 20,000; 30,000; or 50,000 μg , preferably from about 500 μg to about 50,000 μg per 70 kilogram patient. Initial doses followed by boosting doses at established intervals, *e.g.*, from four weeks to six months, may be required, possibly for a prolonged period of time to effectively immunize an individual. Administration should

5 continue until at least clinical symptoms or laboratory tests indicate that the tumor has been eliminated or that the tumor cell burden has been substantially reduced and for a period thereafter. The dosages, routes of administration, and dose schedules are adjusted in accordance with methodologies known in the art.

The pharmaceutical compositions for therapeutic treatment are intended for

10 parenteral, topical, oral, intrathecal, or local administration. Preferably, the pharmaceutical compositions are administered parentally, *e.g.*, intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A

15 variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The

20 compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

25 The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

A human unit dose form of the peptide composition is typically included in a

30 pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see, e.g., Remington's Pharmaceutical Sciences*, 17th Edition, A. Gennaro, Editor, Mack Publishing Co., Easton, Pennsylvania, 1985).

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For targeting cells of the immune system, a ligand to be incorporated into the liposome can include, e.g., antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, *etc.* in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are

the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal delivery.

IV.M. Kits

The peptide and nucleic acid compositions of this invention can be provided in kit form together with instructions for vaccine administration. Typically the kit would include desired peptide compositions in a container, preferably in unit dosage form and instructions for administration. An alternative kit would include a minigene construct with desired nucleic acids of the invention in a container, preferably in unit dosage form together with instructions for administration. Lymphokines such as IL-2 or IL-12 may also be included in the kit. Other kit components that may also be desirable include, for example, a sterile syringe, booster dosages, and other desired excipients.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of non-critical parameters that can be changed or modified to yield alternative embodiments in accordance with the invention.

V. EXAMPLES

The following examples illustrate identification, selection, and use of immunogenic Class I and Class II peptide epitopes for inclusion in vaccine compositions.

Example 1. HLA Class I and Class II Binding Assays

The following example of peptide binding to HLA molecules demonstrates quantification of binding affinities of HLA class I and class II peptides. Binding assays can be performed with peptides that are either motif-bearing or not motif-bearing.

Epstein-Barr virus (EBV)-transformed homozygous cell lines, fibroblasts, CIR, or 721.221-transfectants were used as sources of HLA class I molecules. These cells were maintained *in vitro* by culture in RPMI 1640 medium supplemented with 2mM L-

glutamine (GIBCO, Grand Island, NY), 50 μ M 2-ME, 100 μ g/ml of streptomycin, 100U/ml of penicillin (Irvine Scientific) and 10% heat-inactivated FCS (Irvine Scientific, Santa Ana, CA). Cells were grown in 225-cm² tissue culture flasks or, for large-scale cultures, in roller bottle apparatuses. The specific cell lines routinely used for purification of MHC class I and class II molecules are listed in Table XXIV.

Cell lysates were prepared and HLA molecules purified in accordance with disclosed protocols (Sidney *et al.*, *Current Protocols in Immunology* 18.3.1 (1998); Sidney, *et al.*, *J. Immunol.* 154:247 (1995); Sette, *et al.*, *Mol. Immunol.* 31:813 (1994)). Briefly, cells were lysed at a concentration of 10⁸ cells/ml in 50 mM Tris-HCl, pH 8.5, containing 1% Nonidet P-40 (Fluka Biochemika, Buchs, Switzerland), 150 mM NaCl, 5 mM EDTA, and 2 mM PMSF. Lysates were cleared of debris and nuclei by centrifugation at 15,000 x g for 30min.

HLA molecules were purified from lysates by affinity chromatography. Lysates prepared as above were passed twice through two pre-columns of inactivated Sepharose CL4-B and protein A-Sepharose. Next, the lysate was passed over a column of Sepharose CL-4B beads coupled to an appropriate antibody. The antibodies used for the extraction of HLA from cell lysates are listed in Table XXV. The anti-HLA column was then washed with 10-column volumes of 10mM Tris-HCL, pH 8.0, in 1% NP-40, PBS, 2-column volumes of PBS, and 2-column volumes of PBS containing 0.4% n-octylglucoside. Finally, MHC molecules were eluted with 50mM diethylamine in 0.15M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, was added to the eluate to reduce the pH to ~8.0. Eluates were then concentrated by centrifugation in Centriprep 30 concentrators at 2000 rpm (Amicon, Beverly, MA). Protein content was evaluated by a BCA protein assay (Pierce Chemical Co., Rockford, IL) and confirmed by SDS-PAGE.

A detailed description of the protocol utilized to measure the binding of peptides to Class I and Class II MHC has been published (Sette *et al.*, *Mol. Immunol.* 31:813, 1994; Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998). Briefly, purified MHC molecules (5 to 500nM) were incubated with various unlabeled peptide inhibitors and 1-10nM ¹²⁵I-radiolabeled probe peptides for 48h in PBS containing 0.05% Nonidet P-40 (NP40) (or 20% w/v digitonin for H-2 IA assays) in the presence of a protease inhibitor cocktail. The final concentrations of protease inhibitors (each from CalBioChem, La Jolla, CA) were 1 mM

PMSF, 1.3 nM 1.10 phenanthroline, 73 μ M pepstatin A, 8mM EDTA, 6mM N-ethylmaleimide (for Class II assays), and 200 μ M N alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK). All assays were performed at pH 7.0 with the exception of DRB1*0301, which was performed at pH 4.5, and DRB1*1601 (DR2w21 β ₁) and DRB4*0101

- 5 (DRw53), which were performed at pH 5.0. pH was adjusted as described elsewhere (see Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998).

- Following incubation, MHC-peptide complexes were separated from free peptide by gel filtration on 7.8 mm x 15 cm TSK200 columns (TosoHaas 16215, 10 Montgomeryville, PA), eluted at 1.2 mls/min with PBS pH 6.5 containing 0.5% NP40 and 0.1% NaN₃. Because the large size of the radiolabeled peptide used for the DRB1*1501 (DR2w2 β ₁) assay makes separation of bound from unbound peaks more difficult under these conditions, all DRB1*1501 (DR2w2 β ₁) assays were performed using a 7.8mm x 30cm TSK2000 column eluted at 0.6 mls/min. The eluate from the TSK columns was 15 passed through a Beckman 170 radioisotope detector, and radioactivity was plotted and integrated using a Hewlett-Packard 3396A integrator, and the fraction of peptide bound was determined.

- Radiolabeled peptides were iodinated using the chloramine-T method. Representative radiolabeled probe peptides utilized in each assay, and its assay specific 20 IC₅₀ nM, are summarized in Tables IV and V. Typically, in preliminary experiments, each MHC preparation was titrated in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays were performed using these HLA concentrations.

- 25 Since under these conditions [label]<[HLA] and IC₅₀≥[HLA], the measured IC₅₀ values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 μ g/ml to 1.2 ng/ml, and are tested in two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide 30 by dividing the IC₅₀ of a positive control for inhibition by the IC₅₀ for each tested peptide (typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values can subsequently be converted back into IC₅₀ nM values by dividing the IC₅₀ nM of the

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positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation has proven to be the most accurate and consistent for comparing peptides that have been tested on different days, or with different lots of purified MHC.

5 Because the antibody used for HLA-DR purification (LB3.1) is α -chain specific, β_1 molecules are not separated from β_3 (and/or β_4 and β_5) molecules. The β_1 specificity of the binding assay is obvious in the cases of DRB1*0101 (DR1), DRB1*0802 (DR8w2), and DRB1*0803 (DR8w3), where no β_3 is expressed. It has also been demonstrated for DRB1*0301 (DR3) and DRB3*0101 (DR52a), DRB1*0401 (DR4w4), DRB1*0404
10 (DR4w14), DRB1*0405 (DR4w15), DRB1*1101 (DR5), DRB1*1201 (DR5w12), DRB1*1302 (DR6w19) and DRB1*0701 (DR7). The problem of β chain specificity for DRB1*1501 (DR2w2 β_1), DRB5*0101 (DR2w2 β_2), DRB1*1601 (DR2w21 β_1), DRB5*0201 (DR51Dw21), and DRB4*0101 (DRw53) assays is circumvented by the use of fibroblasts. Development and validation of assays with regard to DR β molecule
15 specificity have been described previously (*see, e.g., Southwood et al., J. Immunol.* 160:3363-3373, 1998).

Binding assays as outlined above may be used to analyze supermotif and/or motif-bearing epitopes as, for example, described in Example 2.

20 Example 2. Identification of HLA Supermotif- and Motif-Bearing CTL Candidate Epitopes

Vaccine compositions of the invention may include multiple epitopes that comprise multiple HLA supermotifs or motifs to achieve broad population coverage. This example illustrates the identification of supermotif- and motif-bearing epitopes for
25 the inclusion in such a vaccine composition. Calculation of population coverage is performed using the strategy described below.

Computer searches and algorithms for identification of supermotif and/or motif-bearing epitopes

30 The searches performed to identify the motif-bearing peptide sequences in Examples 2 and 5 employed protein sequence data for the tumor-associated antigen p53.

Computer searches for epitopes bearing HLA Class I or Class II supermotifs or motifs were performed as follows. All translated protein sequences were analyzed using

a text string search software program, e.g., MotifSearch 1.4 (D. Brown, San Diego) to identify potential peptide sequences containing appropriate HLA binding motifs; alternative programs are readily produced in accordance with information in the art in view of the motif/supermotif disclosure herein. Furthermore, such calculations can be made mentally. Identified A2-, A3-, and DR-supermotif sequences were scored using polynomial algorithms to predict their capacity to bind to specific HLA-Class I or Class II molecules. These polynomial algorithms take into account both extended and refined motifs (that is, to account for the impact of different amino acids at different positions), and are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA molecule interactions can be approximated as a linear polynomial function of the type:

$$“\Delta G” = a_{1i} \times a_{2i} \times a_{3i} \dots \times a_{ni}$$

where a_{ji} is a coefficient which represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. The crucial assumption of this method is that the effects at each position are essentially independent of each other (i.e., independent binding of individual side-chains). When residue j occurs at position i in the peptide, it is assumed to contribute a constant amount j_i to the free energy of binding of the peptide irrespective of the sequence of the rest of the peptide. This assumption is justified by studies from our laboratories that demonstrated that peptides are bound to MHC and recognized by T cells in essentially an extended conformation (data omitted herein).

The method of derivation of specific algorithm coefficients has been described in Gulukota *et al.*, *J. Mol. Biol.* 267:1258-126, 1997; (see also Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; and Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). Briefly, for all i positions, anchor and non-anchor alike, the geometric mean of the average relative binding (ARB) of all peptides carrying j is calculated relative to the remainder of the group, and used as the estimate of j_i . For Class II peptides, if multiple alignments are possible, only the highest scoring alignment is utilized, following an iterative procedure. To calculate an algorithm score of a given peptide in a test set, the ARB values corresponding to the sequence of the peptide are multiplied. If this product exceeds a chosen threshold, the peptide is predicted to bind. Appropriate thresholds are chosen as a function of the degree of stringency of prediction desired.

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Selection of HLA-A2 supertype cross-reactive peptides

The complete protein sequence from p53 was scanned, utilizing motif identification software, to identify 8-, 9-, 10-, and 11-mer sequences containing the HLA-A2-supermotif main anchor specificity.

5 A total of 149 HLA-A2 supermotif-positive sequences were identified and corresponding peptides synthesized. These 149 peptides were then tested for their capacity to bind purified HLA-A*0201 molecules *in vitro* (HLA-A*0201 is considered a prototype A2 supertype molecule). Fourteen of the peptides bound A*0201 with IC₅₀ values ≤500 nM.

10 The fourteen A*0201-binding peptides were subsequently tested for the capacity to bind to additional A2-supertype molecules (A*0202, A*0203, A*0206, and A*6802). As shown in Table XXVI, 10 of the 14 peptides were found to be A2-supertype cross-reactive binders, binding at least three of the five A2-supertype alleles tested. One of the peptides was selected for further evaluation.

15

Selection of HLA-A3 supermotif-bearing epitopes

The protein sequences scanned above are also examined for the presence of peptides with the HLA-A3-supermotif primary anchors using methodology similar to that performed to identify HLA-A2 supermotif-bearing epitopes.

20 Peptides corresponding to the supermotif-bearing sequences are then synthesized and tested for binding to HLA-A*0301 and HLA-A*1101 molecules, the two most prevalent A3-supertype alleles. The peptides that are found to bind one of the two alleles with binding affinities of ≤500 nM are then tested for binding cross-reactivity to the other common A3-supertype alleles (A*3101, A*3301, and A*6801) to identify those that can
25 bind at least three of the five HLA-A3-supertype molecules tested.

Selection of HLA-B7 supermotif bearing epitopes

30 The same target antigen protein sequences are also analyzed to identify HLA-B7-supermotif-bearing sequences. The corresponding peptides are then synthesized and tested for binding to HLA-B*0702, the most common B7-supertype allele (*i.e.*, the prototype B7 supertype allele). Those peptides that bind B*0702 with IC₅₀ of ≤500 nM are then tested for binding to other common B7-supertype molecules (B*3501, B*5101,

B*5301, and B*5401) to identify those peptides that are capable of binding to three or more of the five B7-supertype alleles tested.

Selection of A1 and A24 motif-bearing epitopes

- 5 To further increase population coverage, HLA-A1 and -A24 epitopes can also be incorporated into potential vaccine constructs. An analysis of the protein sequence data from the target antigens utilized above can also be performed to identify HLA-A1- and A24-motif-containing conserved sequences.

10 Example 3. Confirmation of Immunogenicity

One of the cross-reactive candidate CTL A2-supermotif-bearing peptides identified in Example 2 was selected for *in vitro* immunogenicity testing. Testing was performed using the following methodology:

15 **Target Cell Lines for Cellular Screening:**

- The .221A2.1 cell line, produced by transferring the HLA-A2.1 gene into the HLA-A, -B, -C null mutant human B-lymphoblastoid cell line 721.221, was used as the peptide-loaded target to measure activity of HLA-A2.1-restricted CTL. The breast tumor line BT549 was obtained from the American Type Culture Collection (ATCC)
- 20 (Rockville, MD). The Saos-2/175 (Saos-2 transfected with the p53 gene containing a mutation at position 175) was obtained from Dr. Levine, Princeton University, Princeton, NJ. The cell lines that were obtained from ATCC were maintained under the culture conditions recommended by the supplier. All other cell lines were grown in RPMI-1640 medium supplemented with antibiotics, sodium pyruvate, nonessential amino acids and
- 25 10% (v/v) heat inactivated FCS. The p53 tumor targets were treated with 20 ng/ml IFN γ and 3 ng/ml TNF α for 24 hours prior to use as targets in the ^{51}Cr release and *in situ* IFN γ assays (see, e.g., Theobald *et al.*, *Proc. Natl. Acad. Sci. USA* 92:11993, 1995).

Primary CTL Induction Cultures:

- 30 *Generation of Dendritic Cells (DC):* PBMCs were thawed in RPMI with 30 $\mu\text{g/ml}$ DNase, washed twice and resuspended in complete medium (RPMI-1640 plus 5% AB human serum, non-essential amino acids, sodium pyruvate, L-glutamine and penicillin/streptomycin). The monocytes were purified by plating 10×10^6 PBMC/well

in a 6-well plate. After 2 hours at 37°C, the non-adherent cells were removed by gently shaking the plates and aspirating the supernatants. The wells were washed a total of three times with 3 ml RPMI to remove most of the non-adherent and loosely adherent cells. Three ml of complete medium containing 50 ng/ml of GM-CSF and 1,000 U/ml of IL-4 were then added to each well. DC were used for CTL induction cultures following 7 days of culture.

Induction of CTL with DC and Peptide: CD8⁺ T-cells were isolated by positive selection with Dynal immunomagnetic beads (Dynabeads® M-450) and the detachabead® reagent. Typically about 200-250x10⁶ PBMC were processed to obtain 24x10⁶ CD8⁺ T-cells (enough for a 48-well plate culture). Briefly, the PBMCs were thawed in RPMI with 30µg/ml DNase, washed once with PBS containing 1% human AB serum and resuspended in PBS/1% AB serum at a concentration of 20x10⁶ cells/ml. The magnetic beads were washed 3 times with PBS/AB serum, added to the cells (140µl beads/20x10⁶ cells) and incubated for 1 hour at 4°C with continuous mixing. The beads and cells were washed 4x with PBS/AB serum to remove the nonadherent cells and resuspended at 100x10⁶ cells/ml (based on the original cell number) in PBS/AB serum containing 100µl/ml detachabead® reagent and 30µg/ml DNase. The mixture is incubated for 1 hour at room temperature with continuous mixing. The beads were washed again with PBS/AB/DNase to collect the CD8⁺ T-cells. The DC were collected and centrifuged at 1300 rpm for 5-7 minutes, washed once with PBS with 1% BSA, counted and pulsed with 40µg/ml of peptide at a cell concentration of 1-2x10⁶/ml in the presence of 3µg/ml β₂-microglobulin for 4 hours at 20°C. The DC were then irradiated (4,200 rads), washed 1 time with medium and counted again.

Setting up induction cultures: 0.25 ml cytokine-generated DC (@1x10⁵ cells/ml) were co-cultured with 0.25ml of CD8⁺ T-cells (@2x10⁶ cell/ml) in each well of a 48-well plate in the presence of 10 ng/ml of IL-7. rHuman IL10 was added the next day at a final concentration of 10 ng/ml and rhuman IL2 was added 48 hours later at 10IU/ml.

Restimulation of the induction cultures with peptide-pulsed adherent cells: Seven and fourteen days after the primary induction the cells were restimulated with peptide-pulsed adherent cells. The PBMCs were thawed and washed twice with RPMI and DNase. The cells were resuspended at 5x10⁶ cells/ml and irradiated at ~4200 rads. The PBMCs were plated at 2x10⁶ in 0.5ml complete medium per well and incubated for 2 hours at 37°C. The plates were washed twice with RPMI by tapping the plate gently to remove the nonadherent cells and the adherent cells pulsed with 10µg/ml of peptide in the

presence of 3 $\mu\text{g/ml}$ B_2 microglobulin in 0.25ml RPMI/5%AB per well for 2 hours at 37°C. Peptide solution from each well was aspirated and the wells were washed once with RPMI. Most of the media was aspirated from the induction cultures (CD8+ cells) and brought to 0.5 ml with fresh media. The cells were then transferred to the wells containing the peptide-pulsed adherent cells. Twenty four hours later rhuman IL10 was added at a final concentration of 10ng/ml and rhuman IL2 was added the next day and again 2-3 days later at 50IU/ml (Tsai *et al.*, *Critical Reviews in Immunology* 18(1-2):65-75, 1998). Seven days later the cultures were assayed for CTL activity in a ^{51}Cr release assay. In some experiments the cultures were assayed for peptide-specific recognition in the in situ IFN γ ELISA at the time of the second restimulation followed by assay of endogenous recognition 7 days later. After expansion, activity was measured in both assays for a side by side comparison.

Measurement of CTL lytic activity by ^{51}Cr release.

Seven days after the second restimulation, cytotoxicity was determined in a standard (5hr) ^{51}Cr release assay by assaying individual wells at a single E:T. Peptide-pulsed targets were prepared by incubating the cells with 10 $\mu\text{g/ml}$ peptide overnight at 37°C.

Adherent target cells were removed from culture flasks with trypsin-EDTA. Target cells were labelled with 200 μCi of ^{51}Cr sodium chromate (Dupont, Wilmington, DE) for 1 hour at 37°C. Labelled target cells are resuspended at 10^6 per ml and diluted 1:10 with K562 cells at a concentration of $3.3 \times 10^6/\text{ml}$ (an NK-sensitive erythroblastoma cell line used to reduce non-specific lysis). Target cells (100 μl) and 100 μl of effectors were plated in 96 well round-bottom plates and incubated for 5 hours at 37°C. At that time, 100 μl of supernatant were collected from each well and percent lysis was determined according to the formula: $[(\text{cpm of the test sample} - \text{cpm of the spontaneous } ^{51}\text{Cr release sample}) / (\text{cpm of the maximal } ^{51}\text{Cr release sample} - \text{cpm of the spontaneous } ^{51}\text{Cr release sample})] \times 100$. Maximum and spontaneous release were determined by incubating the labelled targets with 1% Triton X-100 and media alone, respectively. A positive culture was defined as one in which the specific lysis (sample-background) was 10% or higher in the case of individual wells and was 15% or more at the 2 highest E:T ratios when expanded cultures were assayed.

***In situ* Measurement of Human IFN γ Production as an Indicator of Peptide-specific and Endogenous Recognition**

- Immulon 2 plates were coated with mouse anti-human IFN γ monoclonal antibody (4 μ g/ml 0.1M NaHCO₃, pH8.2) overnight at 4°C. The plates were washed with Ca²⁺, Mg²⁺-free PBS/0.05% Tween 20 and blocked with PBS/10% FCS for 2 hours, after which the CTLs (100 μ l/well) and targets (100 μ l/well) were added to each well, leaving empty wells for the standards and blanks (which received media only). The target cells, either peptide-pulsed or endogenous targets, were used at a concentration of 1×10^6 cells/ml. The plates were incubated for 48 hours at 37°C with 5% CO₂.
- Recombinant human IFN γ was added to the standard wells starting at 400 pg or 1200pg/100 μ l/well and the plate incubated for 2 hours at 37°C. The plates were washed and 100 μ l of biotinylated mouse anti-human IFN γ monoclonal antibody (4 μ g/ml in PBS/3%FCS/0.05% Tween 20) were added and incubated for 2 hours at room temperature. After washing again, 100 μ l HRP-streptavidin were added and incubated for 1 hour at room temperature. The plates were then washed 6x with wash buffer, 100 μ l/well developing solution (TMB 1:1) were added, and the plates allowed to develop for 5-15 minutes. The reaction was stopped with 50 μ l/well 1M H₃PO₄ and read at OD450. A culture was considered positive if it measured at least 50 pg of IFN γ /well above background and was twice the background level of expression.
- CTL Expansion.** Those cultures that demonstrated specific lytic activity against peptide-pulsed targets and/or tumor targets were expanded over a two week period with anti-CD3. Briefly, 5×10^4 CD8+ cells were added to a T25 flask containing the following: 1×10^6 irradiated (4,200 rad) PBMC (autologous or allogeneic) per ml, 2×10^5 irradiated (8,000 rad) EBV- transformed cells per ml, and OKT3 (anti-CD3) at 30ng per ml in RPMI-1640 containing 10% (v/v) human AB serum, non-essential amino acids, sodium pyruvate, 25 μ M 2-mercaptoethanol, L-glutamine and penicillin/streptomycin. rHuman IL2 was added 24 hours later at a final concentration of 200IU/ml and every 3 days thereafter with fresh media at 50IU/ml. The cells were split if the cell concentration exceeded 1×10^6 /ml and the cultures were assayed between days 13 and 15 at E:T ratios of 30, 10, 3 and 1:1 in the ⁵¹Cr release assay or at 1×10^6 /ml in the *in situ* IFN γ assay using the same targets as before the expansion.

Immunogenicity of A2 supermotif-bearing peptides

The A2-supermotif cross-reactive binding peptide that was selected for further evaluation was tested in the cellular assay for the ability to induce peptide-specific CTL in normal individuals. In this analysis, a peptide was considered to be an epitope if it induced peptide-specific CTLs in at least 2 donors (unless otherwise noted) and if those CTLs also recognized the endogenously expressed peptide. The candidate peptide induced peptide-specific CTLs in only one donor and further analysis demonstrated that no recognition of endogenously expressed p53 was observed (Table XXVII).

10 *Evaluation of A*03/A11 immunogenicity*

HLA-A3 supermotif-bearing cross-reactive binding peptides are also evaluated for immunogenicity using methodology analogous for that used to evaluate the immunogenicity of the HLA-A2 supermotif peptides.

15 *Evaluation of B7 immunogenicity*

Immunogenicity screening of the B7-supertype cross-reactive binding peptides identified in Example 2 are evaluated in a manner analogous to the evaluation of A2-and A3-supermotif-bearing peptides.

20 Example 4. Implementation of the Extended Supermotif to Improve the Binding Capacity of Native Epitopes by Creating Analogs

HLA motifs and supermotifs (comprising primary and/or secondary residues) are useful in the identification and preparation of highly cross-reactive native peptides, as demonstrated herein. Moreover, the definition of HLA motifs and supermotifs also allows one to engineer highly cross-reactive epitopes by identifying residues within a native peptide sequence which can be analogued, or “fixed” to confer upon the peptide certain characteristics, e.g. greater cross-reactivity within the group of HLA molecules that comprise a supertype, and/or greater binding affinity for some or all of those HLA molecules. Examples of analog peptides that exhibit modulated binding affinity are set forth in this example.

Analoguing at Primary Anchor Residues

Peptide engineering strategies were implemented to further increase the cross-reactivity of the epitopes identified above. On the basis of the data disclosed, e.g., in

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related and co-pending U.S.S.N 09/226,775, the main anchors of A2-supermotif-bearing peptides are altered, for example, to introduce a preferred L, I, V, or M at position 2, and I or V at the C-terminus.

Peptides that exhibit at least weak A*0201 binding (IC_{50} of 5000 nM or less), and carrying suboptimal anchor residues at either position 2, the C-terminal position, or both, can be fixed by introducing canonical substitutions (L at position 2 and V at the C-terminus). Those analogued peptides that show at least a three-fold increase in A*0201 binding and bind with an IC_{50} of 500 nM, or less were then tested for A2 cross-reactive binding along with their wild-type (WT) counterparts. Analogued peptides that bind at least three of the five A2 supertype alleles were then selected for cellular screening analysis.

Additionally, the selection of analogs for cellular screening analysis was further restricted by the capacity of the WT parent peptide to bind at least weakly, *i.e.*, bind at an IC_{50} of 5000nM or less, to three of more A2 supertype alleles. The rationale for this requirement is that the WT peptides must be present endogenously in sufficient quantity to be biologically relevant. Analogued peptides have been shown to have increased immunogenicity and cross-reactivity by T cells specific for the WT epitope (*see, e.g.*, Parkhurst *et al.*, *J. Immunol.* 157:2539, 1996; and Pogue *et al.*, *Proc. Natl. Acad. Sci. USA* 92:8166, 1995).

In the cellular screening of these peptide analogs, it is important to demonstrate that analog-specific CTLs are also able to recognize the wild-type peptide and, when possible, tumor targets that endogenously express the epitope.

Nineteen p53 peptides met the criteria for analoguing at primary anchor residues by introducing a canonical substitution: these peptides showed at least weak A*0201 binding (IC_{50} of 5000 nM or less) and carried suboptimal anchor residues. These peptides were analogued and tested for binding to A*0201 (Table XXII). Eighteen of the analog peptides representing 12 epitopes were tested then for cross-reactive binding. Eleven of these analogs exhibited improved crossbinding capability (Table XXVIII).

The 11 analog peptides were additionally evaluated for *in vitro* immunogenicity using cellular screening. In the case of p53, it is important to demonstrate induction of peptide-specific CTL and to then use those cells to identify an endogenous tumor target. Each assay also included the epitope HBVc.18 as an internal control. When peptide p53.139L2 was used to induce CTLs in a normal donor, measurable CTL activity was observed in 3 of 48 wells. Each well was expanded and two weeks later, reassayed

against the induction peptide and the appropriate wildtype peptide. The p53.139L2-specific CTLs maintained their lytic activity. Additionally, two of these cultures recognized the parental, wildtype peptide.

These cells were then used to assess endogenous target cell lines. Numerous HLA-A2⁺, p53-expressing tumor lines have been described (*see, e.g.,* Theobald *et al., Proc. Natl. Acad. Sci. USA*, 92:11993, 1995) and were readily available. These included BT549, a breast infiltrating ductal carcinoma line, and Saos-2/175, a transfected cell line. Saos-2, an osteogenic sarcoma that is HLA-A2⁺ and p53⁻, was used as the negative control cell line. The results of the analysis showed that two individual CTL cultures to peptide p53.139L2 demonstrated significant lysis of the endogenous target BT549.

Of the available analogs tested, ten induced a peptide-specific response in 2 or more donors. Of these 10, 8 generated CTLs that recognized the wild-type peptide and 4 of these recognized tumor targets (Table XXIX). Two of these analogs, p53.139L2 and p53.139L2B3, differed only at position three. The assay results indicated that the CTLs to p53.139L2B3 recognized the target cells pulsed with wild-type peptide as well as the analog, and also recognized the tumor target cell line BT549. Another analog peptide, p53.149M2, also demonstrated significant improvement over the wildtype peptide. Six individual wells met the criteria for a positive response and the cells cultured in one of the wells maintained that activity upon expansion of the population. All the CTLs generated recognized the wildtype peptide and were also able to lyse the Saos-2/175 transfected cell line, which expresses p53. A fourth epitope, p53.69L2V8, also demonstrated recognition of the wildtype peptide.

Using methodology similar to that used to develop HLA-A2 analogs, analogs of HLA-A3 and HLA-B7 supermotif-bearing epitopes are also generated. For example, peptides binding at least weakly to 3/5 of the A3-supertype molecules may be engineered at primary anchor residues to possess a preferred residue (V, S, M, or A) at position 2. The analog peptides are then tested for the ability to bind A*03 and A*11 (prototype A3 supertype alleles). Those peptides that demonstrate ≤ 500 nM binding capacity are then tested for A3-supertype cross-reactivity. B7 supermotif-bearing peptides may, for example, be engineered to possess a preferred residue (V, I, L, or F) at the C-terminal primary anchor position, as demonstrated by Sidney *et al. (J. Immunol.* 157:3480-3490, 1996) and tested for binding to B7 supertype alleles.

Analoguing at Secondary Anchor Residues

Moreover, HLA supermotifs are of value in engineering highly cross-reactive peptides and/or peptides that bind HLA molecules with increased affinity by identifying particular residues at secondary anchor positions that are associated with such properties.

5 For example, the binding capacity of a B7 supermotif-bearing peptide representing a discreet single amino acid substitution at position 1 can be analyzed. A peptide can, for example, be analogued to substitute L with F at position 1 and subsequently be evaluated for increased binding affinity/ and or increased cross-reactivity. This procedure will identify analogued peptides with modulated binding affinity.

10 Engineered analogs with sufficiently improved binding capacity or cross-reactivity are tested for immunogenicity as above.

Other analoguing strategies

15 Another form of peptide analoguing, unrelated to the anchor positions, involves the substitution of a cysteine with α -amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substitution of α -amino butyric acid for cysteine not only alleviates this problem, but has been shown to improve binding and crossbinding capabilities in some instances (*see, e.g.*, the review by Sette *et al.*, In: 20 Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

In conclusion, these data demonstrate that by the use of even single amino acid substitutions, it is possible to increase the binding affinity and/or cross-reactivity of peptide ligands for HLA supertype molecules.

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Example 5. Identification of peptide epitope sequences with HLA-DR binding motifs

Peptide epitopes bearing an HLA class II supermotif or motif may also be identified as outlined below using methodology similar to that described in Examples 1-3.

30 *Selection of HLA-DR-supermotif-bearing epitopes*

To identify HLA class II HTL epitopes, the p53 protein sequence was analyzed for the presence of sequences bearing an HLA-DR-motif or supermotif. Specifically, 15-

mer sequences were selected comprising a DR-supermotif, further comprising a 9-mer core, and three-residue N- and C-terminal flanking regions (15 amino acids total).

Protocols for predicting peptide binding to DR molecules have been developed (Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). These protocols, specific for individual DR molecules, allow the scoring, and ranking, of 9-mer core regions. Each protocol not only scores peptide sequences for the presence of DR-supermotif primary anchors (i.e., at position 1 and position 6) within a 9-mer core, but additionally evaluates sequences for the presence of secondary anchors. Using allele specific selection tables (see, e.g., Southwood *et al.*, *ibid.*), it has been found that these protocols efficiently select peptide sequences with a high probability of binding a particular DR molecule. Additionally, it has been found that performing these protocols in tandem, specifically those for DR1, DR4w4, and DR7, can efficiently select DR cross-reactive peptides.

The p53-derived peptides identified above were tested for their binding capacity for various common HLA-DR molecules. All peptides were initially tested for binding to the DR molecules in the primary panel: DR1, DR4w4, and DR7. Peptides binding at least 2 of these 3 DR molecules with an IC₅₀ value of 1000 nM or less, were then tested for binding to DRB5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302. Peptides were considered to be cross-reactive DR supertype binders if they bound at an IC₅₀ value of 1000 nM or less to at least 5 of the 8 alleles tested.

Following the strategy outlined above, 50 DR supermotif-bearing sequences were identified within the p53 protein sequence. Of those, 6 scored positive in 2 of the 3 combined DR 147 algorithms. These peptides were synthesized and tested for binding to HLA-DRB1*0101, DRB1*0401, DRB1*0701 with 3, 2, and 2 peptides binding ≤1000 nM, respectively. Of the 6 peptides tested for binding to these primary HLA molecules, 2 bound at least 2 of the 3 alleles (Table XXX).

These 2 peptides were then tested for binding to secondary DR supertype alleles: DRB5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302. Both peptides bound at least 5 of the 8 alleles tested, of which 8 occurred in distinct, non-overlapping regions (Table XXXI).

30

Selection of DR3 motif peptides

Because HLA-DR3 is an allele that is prevalent in Caucasian, Black, and Hispanic populations, DR3 binding capacity is an important criterion in the selection of HTL

epitopes. However, data generated previously indicated that DR3 only rarely cross-reacts with other DR alleles (Sidney *et al.*, *J. Immunol.* 149:2634-2640, 1992; Geluk *et al.*, *J. Immunol.* 152:5742-5748, 1994; Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998).

This is not entirely surprising in that the DR3 peptide-binding motif appears to be distinct from the specificity of most other DR alleles. For maximum efficiency in developing vaccine candidates it would be desirable for DR3 motifs to be clustered in proximity with DR supermotif regions. Thus, peptides shown to be candidates may also be assayed for their DR3 binding capacity. However, in view of the distinct binding specificity of the DR3 motif, peptides binding only to DR3 can also be considered as candidates for inclusion in a vaccine formulation.

To efficiently identify peptides that bind DR3, the p53 protein sequence was analyzed for conserved sequences carrying one of the two DR3 specific binding motifs (Table III) reported by Geluk *et al.* (*J. Immunol.* 152:5742-5748, 1994). Sixteen motif-positive peptides were identified. The corresponding peptides were then synthesized and tested for the ability to bind DR3 with an affinity of ≤ 1000 nM. No peptides were identified that met this binding criterion (Table XXXII), and thereby qualify as HLA class II high affinity binders.

In summary, 2 DR supertype cross-reactive binding peptides were identified from the p53 protein sequence (Table XXXIII).

Similarly to the case of HLA class I motif-bearing peptides, the class II motif-bearing peptides may be analogued to improve affinity or cross-reactivity. For example, aspartic acid at position 4 of the 9-mer core sequence is an optimal residue for DR3 binding, and substitution for that residue may improve DR 3 binding.

Example 6. Immunogenicity of HTL epitopes

This example determines immunogenic DR supermotif- and DR3 motif-bearing epitopes among those identified using the methodology in Example 5. Immunogenicity of HTL epitopes are evaluated in a manner analogous to the determination of immunogenicity of CTL epitopes by assessing the ability to stimulate HTL responses and/or by using appropriate transgenic mouse models. Immunogenicity is determined by screening for: 1.) *in vitro* primary induction using normal PBMC or 2.) recall responses from cancer patient PBMCs.

Example 7. Calculation of phenotypic frequencies of HLA-supertypes in various ethnic backgrounds to determine breadth of population coverage

This example illustrates the assessment of the breadth of population coverage of a vaccine composition comprised of multiple epitopes comprising multiple supermotifs and/or motifs.

In order to analyze population coverage, gene frequencies of HLA alleles were determined. Gene frequencies for each HLA allele were calculated from antigen or allele frequencies utilizing the binomial distribution formulae $gf=1-(\text{SQRT}(1-af))$ (see, e.g., Sidney *et al.*, *Human Immunol.* 45:79-93, 1996). To obtain overall phenotypic frequencies, cumulative gene frequencies were calculated, and the cumulative antigen frequencies derived by the use of the inverse formula $[af=1-(1-Cgf)^2]$.

Where frequency data was not available at the level of DNA typing, correspondence to the serologically defined antigen frequencies was assumed. To obtain total potential supertype population coverage no linkage disequilibrium was assumed, and only alleles confirmed to belong to each of the supertypes were included (minimal estimates). Estimates of total potential coverage achieved by inter-loci combinations were made by adding to the A coverage the proportion of the non-A covered population that could be expected to be covered by the B alleles considered (e.g., $\text{total}=A+B*(1-A)$). Confirmed members of the A3-like supertype are A3, A11, A31, A*3301, and A*6801. Although the A3-like supertype may also include A34, A66, and A*7401, these alleles were not included in overall frequency calculations. Likewise, confirmed members of the A2-like supertype family are A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, and A*6901. Finally, the B7-like supertype-confirmed alleles are: B7, B*3501-03, B51, B*5301, B*5401, B*5501-2, B*5601, B*6701, and B*7801 (potentially also B*1401, B*3504-06, B*4201, and B*5602).

Population coverage achieved by combining the A2-, A3- and B7-supertypes is approximately 86% in five major ethnic groups (see Table XXI). Coverage may be extended by including peptides bearing the A1 and A24 motifs. On average, A1 is present in 12% and A24 in 29% of the population across five different major ethnic groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Together, these alleles are represented with an average frequency of 39% in these same ethnic populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95%. An

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analogous approach can be used to estimate population coverage achieved with combinations of class II motif-bearing epitopes.

Example 8. Recognition Of Generation Of Endogenous Processed Antigens After

5 Priming

This example determines that CTL induced by native or analogued peptide epitopes identified and selected as described in Examples 1-6 recognize endogenously synthesized, *i.e.*, native antigens, using a transgenic mouse model.

Effector cells isolated from transgenic mice that are immunized with peptide
10 epitopes (as described, e.g., in Wentworth et al., *Mol. Immunol.* 32:603, 1995), for example HLA-A2 supermotif-bearing epitopes, are re-stimulated *in vitro* using peptide-coated stimulator cells. Six days later, effector cells are assayed for cytotoxicity and the cell lines that contain peptide-specific cytotoxic activity are further re-stimulated. An additional six days later, these cell lines are tested for cytotoxic activity on ⁵¹Cr labeled
15 Jurkat-A2.1/K^b target cells in the absence or presence of peptide, and also tested on ⁵¹Cr labeled target cells bearing the endogenously synthesized antigen, *i.e.* cells that are stably transfected with TAA expression vectors.

The result will demonstrate that CTL lines obtained from animals primed with peptide epitope recognize endogenously synthesized antigen. The choice of transgenic
20 mouse model to be used for such an analysis depends upon the epitope(s) that is being evaluated. In addition to HLA-A*0201/K^b transgenic mice, several other transgenic mouse models including mice with human A11, which may also be used to evaluate A3 epitopes, and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have
25 also been developed, which may be used to evaluate HTL epitopes.

Example 9. Activity Of CTL-HTL Conjugated Epitopes In Transgenic Mice

This example illustrates the induction of CTLs and HTLs in transgenic mice by use of a tumor associated antigen CTL/HTL peptide conjugate whereby the vaccine
30 composition comprises peptides to be administered to a cancer patient. The peptide composition can comprise multiple CTL and/or HTL epitopes and further, can comprise epitopes selected from multiple-tumor associated antigens. The epitopes are identified using methodology as described in Examples 1-6 This analysis demonstrates the enhanced immunogenicity that can be achieved by inclusion of one or more HTL epitopes

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in a vaccine composition. Such a peptide composition can comprise an HTL epitope conjugated to a preferred CTL epitope containing, for example, at least one CTL epitope selected from Tables XXVI, XXVII, XXVIII, or other analogs of that epitope. The HTL epitope is, for example, selected from Table XXXIII. The peptides may be lipidated, if desired.

Immunization procedures: Immunization of transgenic mice is performed as described (Alexander *et al.*, *J. Immunol.* 159:4753-4761, 1997). For example, A2/K^b mice, which are transgenic for the human HLA A2.1 allele and are useful for the assessment of the immunogenicity of HLA-A*0201 motif- or HLA-A2 supermotif-bearing epitopes, are primed subcutaneously (base of the tail) with 0.1 ml of peptide conjugate formulated in saline, or DMSO/saline. Seven days after priming, splenocytes obtained from these animals are restimulated with syngenic irradiated LPS-activated lymphoblasts coated with peptide.

The target cells for peptide-specific cytotoxicity assays are Jurkat cells transfected with the HLA-A2.1/K^b chimeric gene (*e.g.*, Vitiello *et al.*, *J. Exp. Med.* 173:1007, 1991).

In vitro CTL activation: One week after priming, spleen cells (30x10⁶ cells/flask) are co-cultured at 37°C with syngenic, irradiated (3000 rads), peptide coated lymphoblasts (10x10⁶ cells/flask) in 10 ml of culture medium/T25 flask. After six days, effector cells are harvested and assayed for cytotoxic activity.

Assay for cytotoxic activity: Target cells (1.0 to 1.5x10⁶) are incubated at 37°C in the presence of 200 µl of ⁵¹Cr. After 60 minutes, cells are washed three times and resuspended in medium. Peptide is added where required at a concentration of 1 µg/ml. For the assay, 10⁴ ⁵¹Cr-labeled target cells are added to different concentrations of effector cells (final volume of 200 µl) in U-bottom 96-well plates. After a 6 hour incubation period at 37°C, a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = 100 x

(experimental release - spontaneous release)/(maximum release - spontaneous release).

To facilitate comparison between separate CTL assays run under the same conditions, % ⁵¹Cr release data is expressed as lytic units/10⁶ cells. One lytic unit is arbitrarily defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a 6 hour ⁵¹Cr release assay. To obtain specific lytic units/10⁶, the lytic units/10⁶ obtained in the absence of peptide is subtracted from the lytic units/10⁶ obtained in the presence of peptide. For example, if 30% ⁵¹Cr release is obtained at the effector (E): target (T) ratio

of 50:1 (i.e., 5×10^5 effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., 5×10^4 effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: $[(1/50,000) - (1/500,000)] \times 10^6 = 18 \text{ LU}$.

The results are analyzed to assess the magnitude of the CTL responses of animals injected with the immunogenic CTL/HTL conjugate vaccine preparation. The frequency and magnitude of response can also be compared to the CTL response achieved using the CTL epitopes by themselves. Analyses similar to this may be performed to evaluate the immunogenicity of peptide conjugates containing multiple CTL epitopes and/or multiple HTL epitopes. In accordance with these procedures it is found that a CTL response is induced, and concomitantly that an HTL response is induced upon administration of such compositions.

Example 10. Selection of CTL and HTL epitopes for inclusion in a cancer vaccine.

This example illustrates the procedure for the selection of peptide epitopes for vaccine compositions of the invention. The peptides in the composition may be in the form of a nucleic acid sequence, either single or one or more sequences (i.e., minigene) that encodes peptide(s), or may be single and/or polyepitopic peptides.

The following principles are utilized when selecting an array of epitopes for inclusion in a vaccine composition. Each of the following principles are balanced in order to make the selection.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For HLA Class I this includes 3-4 epitopes that come from at least one TAA. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one TAA (*see e.g.*, Rosenberg *et al.*, *Science* 278:1447-1450). Epitopes from one TAA may be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, *e.g.*, in Example 15.

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less.

3.) Sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. For

example, epitopes are selected to provide at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art and discussed herein, can be employed to assess breadth, or redundancy, of population coverage.

4.) When selecting epitopes from cancer-related antigens it is often preferred to select analogs because the patient may have developed tolerance to the native epitope. When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes. Of relevance for infectious disease vaccines (but for cancer-related vaccines as well), are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it.

When providing nested epitopes, a sequence that has the greatest number of epitopes per provided sequence is provided. A limitation on this principle is to avoid providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a longer peptide sequence, such as a sequence comprising nested epitopes, the sequence is screened in order to insure that it does not have pathological or other deleterious biological properties.

5.) When creating a minigene, as disclosed in greater detail in Example 11, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those employed when selecting a peptide comprising nested epitopes. Additionally, however, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide sequence encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, e.g., by motif analysis. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that epitope, which is not present in a native protein sequence. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

Peptide epitopes for inclusion in vaccine compositions are, for example, selected from those listed in Tables XXVI-XXVIII, and XXXIII. A vaccine composition comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response that results in tumor cell killing and reduction of tumor size or mass.

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Example 11. Construction of Minigene Multi-Epitope DNA Plasmids

This example provides general guidance for the construction of a minigene expression plasmid. Minigene plasmids may, of course, contain various configurations of CTL and/or HTL epitopes or epitope analogs as described herein. Expression plasmids have been constructed and evaluated as described, for example, in co-pending U.S.S.N. 09/311,784 filed 5/13/99.

A minigene expression plasmid may include multiple CTL and HTL peptide epitopes. In the present example, HLA-A2, -A3, -B7 supermotif-bearing peptide epitopes and HLA-A1 and -A24 motif-bearing peptide epitopes are used in conjunction with DR supermotif-bearing epitopes and/or DR3 epitopes. Preferred epitopes are identified, for example, in Tables XXVI-XXVIII, and XXXIII. HLA class I supermotif or motif-bearing peptide epitopes derived from multiple TAAs are selected such that multiple supermotifs/motifs are represented to ensure broad population coverage. Similarly, HLA class II epitopes are selected from multiple tumor antigens to provide broad population coverage, *i.e.* both HLA DR-1-4-7 supermotif-bearing epitopes and HLA DR-3 motif-bearing epitopes are selected for inclusion in the minigene construct. The selected CTL and HTL epitopes are then incorporated into a minigene for expression in an expression vector.

This example illustrates the methods to be used for construction of such a minigene-bearing expression plasmid. Other expression vectors that may be used for minigene compositions are available and known to those of skill in the art.

The minigene DNA plasmid contains a consensus Kozak sequence and a consensus murine kappa Ig-light chain signal sequence followed by CTL and/or HTL epitopes selected in accordance with principles disclosed herein. The sequence encodes an open reading frame fused to the Myc and His antibody epitope tag coded for by the pcDNA 3.1 Myc-His vector.

Overlapping oligonucleotides, for example eight oligonucleotides, averaging approximately 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides, Kozak sequence, and signal sequence. The final multiepitope minigene is assembled by extending the overlapping oligonucleotides in three sets of reactions using PCR. A Perkin/Elmer 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing

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temperature (5° below the lowest calculated T_m of each primer pair) for 30 sec, and 72°C for 1 min.

For the first PCR reaction, 5 µg of each of two oligonucleotides are annealed and extended: Oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 µl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM (NH₄)₂SO₄, 20 mM Tris-chloride, pH 8.75, 2 mM MgSO₄, 0.1% Triton X-100, 100 µg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-length dimer products are gel-purified, and two reactions containing the product of 1+2 and 3+4, and the product of 5+6 and 7+8 are mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product for 25 additional cycles. The full-length product is gel-purified and cloned into pCR-blunt (Invitrogen) and individual clones are screened by sequencing.

Example 12. The plasmid construct and the degree to which it induces immunogenicity.

The degree to which the plasmid construct prepared using the methodology outlined in Example 11 is able to induce immunogenicity is evaluated through *in vivo* injections into mice and subsequent *in vitro* assessment of CTL and HTL activity, which are analysed using cytotoxicity and proliferation assays, respectively, as detailed *e.g.*, in U.S.S.N. 09/311,784 filed 5/13/99 and Alexander *et al.*, *Immunity* 1:751-761, 1994.

Alternatively, plasmid constructs can be evaluated *in vitro* by testing for epitope presentation by APC following transduction or transfection of the APC with an epitope-expressing nucleic acid construct. Such a study determines “antigenicity” and allows the use of human APC. The assay determines the ability of the epitope to be presented by the APC in a context that is recognized by a T cell by quantifying the density of epitope-HLA class I complexes on the cell surface. Quantitation can be performed by directly measuring the amount of peptide eluted from the APC (*see, e.g.*, Sijts *et al.*, *J. Immunol.* 156:683-692, 1996; Demotz *et al.*, *Nature* 342:682-684, 1989); or the number of peptide-HLA class I complexes can be estimated by measuring the amount of lysis or lymphokine release induced by infected or transfected target cells, and then determining the concentration of peptide necessary to obtained equivalent levels of lysis or lymphokine release (*see, e.g.*, Kageyama *et al.*, *J. Immunol.* 154:567-576, 1995).

To assess the capacity of the minigene construct (*e.g.*, a pMin minigene construct generated as described in U.S.S.N. 09/311,784) to induce CTLs *in vivo*, HLA-A11/K^b

transgenic mice, for example, are immunized intramuscularly with 100 µg of naked cDNA. As a means of comparing the level of CTLs induced by cDNA immunization, a control group of animals is also immunized with an actual peptide composition that comprises multiple epitopes synthesized as a single polypeptide as they would be encoded
 5 by the minigene.

Splenocytes from immunized animals are stimulated twice with each of the respective compositions (peptide epitopes encoded in the minigene or the polyepitopic peptide), then assayed for peptide-specific cytotoxic activity in a ⁵¹Cr release assay. The results indicate the magnitude of the CTL response directed against the A3-restricted
 10 epitope, thus indicating the *in vivo* immunogenicity of the minigene vaccine and polyepitopic vaccine. It is, therefore, found that the minigene elicits immune responses directed toward the HLA-A3 supermotif peptide epitopes as does the polyepitopic peptide vaccine. A similar analysis is also performed using other HLA-A2 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A2 and HLA-B7 motif or
 15 supermotif epitopes.

To assess the capacity of a class II epitope encoding minigene to induce HTLs *in vivo*, I-A^b restricted mice, for example, are immunized intramuscularly with 100 µg of plasmid DNA. As a means of comparing the level of HTLs induced by DNA immunization, a group of control animals is also immunized with an actual peptide
 20 composition emulsified in complete Freund's adjuvant. CD4⁺ T cells, *i.e.* HTLs, are purified from splenocytes of immunized animals and stimulated with each of the respective compositions (peptides encoded in the minigene). The HTL response is measured using a ³H-thymidine incorporation proliferation assay, (*see, e.g.*, Alexander et al. Immunity 1:751-761, 1994). The results indicate the magnitude of the HTL response,
 25 thus demonstrating the *in vivo* immunogenicity of the minigene.

DNA minigenes, constructed as described in Example 11, may also be evaluated as a vaccine in combination with a boosting agent using a prime boost protocol. The boosting agent may consist of recombinant protein (*e.g.*, Barnett *et al.*, *Aids Res. and Human Retroviruses* 14, Supplement 3:S299-S309, 1998) or recombinant vaccinia, for
 30 example, expressing a minigene or DNA encoding the complete protein of interest (*see, e.g.*, Hanke et al., *Vaccine* 16:439-445, 1998; Sedegah *et al.*, *Proc. Natl. Acad. Sci USA* 95:7648-53, 1998; Hanke and McMichael, *Immunol. Letters* 66:177-181, 1999; and Robinson *et al.*, *Nature Med.* 5:526-34, 1999).

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For example, the efficacy of the DNA minigene may be evaluated in transgenic mice. In this example, A2.1/K^b transgenic mice are immunized IM with 100 µg of the DNA minigene encoding the immunogenic peptides. After an incubation period (ranging from 3-9 weeks), the mice are boosted IP with 10⁷ pfu/mouse of a recombinant vaccinia virus expressing the same sequence encoded by the DNA minigene. Control mice are immunized with 100 µg of DNA or recombinant vaccinia without the minigene sequence, or with DNA encoding the minigene, but without the vaccinia boost. After an additional incubation period of two weeks, splenocytes from the mice are immediately assayed for peptide-specific activity in an ELISPOT assay. Additionally, splenocytes are stimulated *in vitro* with the A2-restricted peptide epitopes encoded in the minigene and recombinant vaccinia, then assayed for peptide-specific activity in an IFN-γ ELISA. It is found that the minigene utilized in a prime-boost mode elicits greater immune responses toward the HLA-A2 supermotif peptides than with DNA alone. Such an analysis is also performed using other HLA-A11 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A3 and HLA-B7 motif or supermotif epitopes.

Example 13. Peptide Composition for Prophylactic Uses

Vaccine compositions of the present invention are used to prevent cancer in persons who are at risk for developing a tumor. For example, a polyepitopic peptide epitope composition (or a nucleic acid comprising the same) containing multiple CTL and HTL epitopes such as those selected in Examples 9 and/or 10, which are also selected to target greater than 80% of the population, is administered to an individual at risk for a cancer, *e.g.*, breast cancer. The composition is provided as a single polypeptide that encompasses multiple epitopes. The vaccine is administered in an aqueous carrier comprised of Freund's Incomplete Adjuvant. The dose of peptide for the initial immunization is from about 1 to about 50,000 µg, generally 100-5,000 µg, for a 70 kg patient. The initial administration of vaccine is followed by booster dosages at 4 weeks followed by evaluation of the magnitude of the immune response in the patient, by techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found to be both safe and efficacious as a prophylaxis against cancer.

Alternatively, the polyepitopic peptide composition can be administered as a nucleic acid in accordance with methodologies known in the art and disclosed herein.

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Example 14. Polyepitopic Vaccine Compositions Derived from Native TAA Sequences

A native TAA polyprotein sequence is screened, preferably using computer algorithms defined for each class I and/or class II supermotif or motif, to identify

5 “relatively short” regions of the polyprotein that comprise multiple epitopes and is preferably less in length than an entire native antigen. This relatively short sequence that contains multiple distinct, even overlapping, epitopes is selected and used to generate a minigene construct. The construct is engineered to express the peptide, which corresponds to the native protein sequence. The “relatively short” peptide is generally

10 less than 1,000, 500, 250 amino acids in length, often less than 100 amino acids in length, preferably less than 75 amino acids in length, and more preferably less than 50 amino acids in length. The protein sequence of the vaccine composition is selected because it has a maximal number of epitopes contained within the sequence, *i.e.*, it has a high concentration of epitopes. As noted herein, epitope motifs may be nested or overlapping

15 (*i.e.*, frame shifted relative to one another). For example, with frame shifted overlapping epitopes, two 9-mer epitopes and one 10-mer epitope can be present in a 10 amino acid peptide. Such a vaccine composition is administered for therapeutic or prophylactic purposes.

The vaccine composition will preferably include, for example, three CTL epitopes

20 and at least one HTL epitope from TAAs. This polyepitopic native sequence is administered either as a peptide or as a nucleic acid sequence which encodes the peptide. Alternatively, an analog can be made of this native sequence, whereby one or more of the epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polyepitopic peptide.

25 The embodiment of this example provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA makeup that is presently unknown.

30 Furthermore, this embodiment (absent analogs) directs the immune response to multiple peptide sequences that are actually present in native TAAs thus avoiding the need to evaluate any junctional epitopes. Lastly, the embodiment provides an economy of scale when producing nucleic acid vaccine compositions.

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Related to this embodiment, computer programs can be derived in accordance with principles in the art, which identify in a target sequence, the greatest number of epitopes per sequence length.

5 Example 15. Polyepitopic Vaccine Compositions Directed To Multiple Tumors

10 The p53 peptide epitopes of the present invention are used in conjunction with peptide epitopes from other target tumor antigens to create a vaccine composition that is useful for the treatment of various types of tumors. For example, a set of TAA epitopes can be selected that allows the targeting of most common epithelial tumors (*see, e.g.,* Kawashima *et al.*, *Hum. Immunol.* 59:1-14, 1998). Such a composition can additionally include epitopes from CEA, HER-2/neu, and MAGE2/3, all of which are expressed to appreciable degrees (20-60%) in frequently found tumors such as lung, breast, and gastrointestinal tumors.

15 The composition can be provided as a single polypeptide that incorporates the multiple epitopes from the various TAAs, or can be administered as a composition comprising one or more discrete epitopes. Alternatively, the vaccine can be administered as a minigene construct or as dendritic cells which have been loaded with the peptide epitopes *in vitro*.

20 Targeting multiple tumor antigens is also important to provide coverage of a large fraction of tumors of any particular type. A single TAA is rarely expressed in the majority of tumors of a given type. For example, approximately 50% of breast tumors express CEA, 20% express MAGE3, and 30% express HER-2/neu. Thus, the use of a single antigen for immunotherapy would offer only limited patient coverage. The combination of the three TAAs, however, would address approximately 70% of breast
25 tumors. A vaccine composition comprising epitopes from multiple tumor antigens also reduces the potential for escape mutants due to loss of expression of an individual tumor antigen.

30 Example 16. Use of peptides to evaluate an immune response

Peptides of the invention may be used to analyze an immune response for the presence of specific CTL or HTL populations directed to a TAA. Such an analysis may be performed using multimeric complexes as described, *e.g.*, by Ogg *et al.*, *Science* 279:2103-2106, 1998 and Greten *et al.*, *Proc. Natl. Acad. Sci. USA* 95:7568-7573, 1998.

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In the following example, peptides in accordance with the invention are used as a reagent for diagnostic or prognostic purposes, not as an immunogen.

In this example, highly sensitive human leukocyte antigen tetrameric complexes ("tetramers") are used for a cross-sectional analysis of, for example, tumor-associated antigen HLA-A*0201-specific CTL frequencies from HLA A*0201-positive individuals at different stages of disease or following immunization using a TAA peptide containing an A*0201 motif. Tetrameric complexes are synthesized as described (Musey *et al.*, *N. Engl. J. Med.* 337:1267, 1997). Briefly, purified HLA heavy chain (A*0201 in this example) and β 2-microglobulin are synthesized by means of a prokaryotic expression system. The heavy chain is modified by deletion of the transmembrane-cytosolic tail and COOH-terminal addition of a sequence containing a BirA enzymatic biotinylation site. The heavy chain, β 2-microglobulin, and peptide are refolded by dilution. The 45-kD refolded product is isolated by fast protein liquid chromatography and then biotinylated by BirA in the presence of biotin (Sigma, St. Louis, Missouri), adenosine 5'triphosphate and magnesium. Streptavidin-phycoerythrin conjugate is added in a 1:4 molar ratio, and the tetrameric product is concentrated to 1 mg/ml. The resulting product is referred to as tetramer-phycoerythrin.

For the analysis of patient blood samples, approximately one million PBMCs are centrifuged at 300g for 5 minutes and resuspended in 50 μ l of cold phosphate-buffered saline. Tri-color analysis is performed with the tetramer-phycoerythrin, along with anti-CD8-Tricolor, and anti-CD38. The PBMCs are incubated with tetramer and antibodies on ice for 30 to 60 min and then washed twice before formaldehyde fixation. Gates are applied to contain >99.98% of control samples. Controls for the tetramers include both A*0201-negative individuals and A*0201-positive uninfected donors. The percentage of cells stained with the tetramer is then determined by flow cytometry. The results indicate the number of cells in the PBMC sample that contain epitope-restricted CTLs, thereby readily indicating the extent of immune response to the TAA epitope, and thus the stage of tumor progression or exposure to a vaccine that elicits a protective or therapeutic response.

30

Example 17. Use of Peptide Epitopes to Evaluate Recall Responses

The peptide epitopes of the invention are used as reagents to evaluate T cell responses, such as acute or recall responses, in patients. Such an analysis may be

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performed on patients who are in remission, have a tumor, or who have been vaccinated with a TAA vaccine.

For example, the class I restricted CTL response of persons who have been vaccinated may be analyzed. The vaccine may be any TAA vaccine. PBMC are
 5 collected from vaccinated individuals and HLA typed. Appropriate peptide epitopes of the invention that, optimally, bear supermotifs to provide cross-reactivity with multiple HLA supertype family members, are then used for analysis of samples derived from individuals who bear that HLA type.

PBMC from vaccinated individuals are separated on Ficoll-Histopaque density
 10 gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 µg/ml), and Hepes (10mM) containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. A synthetic peptide comprising an epitope of the invention is
 15 added at 10 µg/ml to each well and HBV core 128-140 epitope is added at 1 µg/ml to each well as a source of T cell help during the first week of stimulation.

In the microculture format, 4×10^5 PBMC are stimulated with peptide in 8 replicate cultures in 96-well round bottom plate in 100 µl/well of complete RPMI. On days 3 and 10, 100 µl of complete RPMI and 20 U/ml final concentration of rIL-2 are
 20 added to each well. On day 7 the cultures are transferred into a 96-well flat-bottom plate and restimulated with peptide, rIL-2 and 10^5 irradiated (3,000 rad) autologous feeder cells. The cultures are tested for cytotoxic activity on day 14. A positive CTL response requires two or more of the eight replicate cultures to display greater than 10% specific ^{51}Cr release, based on comparison with uninfected control subjects as previously
 25 described (Rehermann, *et al.*, *Nature Med.* 2:1104,1108, 1996; Rehermann *et al.*, *J. Clin. Invest.* 97:1655-1665, 1996; and Rehermann *et al.* *J. Clin. Invest.* 98:1432-1440, 1996).

Target cell lines are autologous and allogeneic EBV-transformed B-LCL that are either purchased from the American Society for Histocompatibility and Immunogenetics (ASHI, Boston, MA) or established from the pool of patients as described (Guilhot, *et al.*
 30 *J. Virol.* 66:2670-2678, 1992).

Cytotoxicity assays are performed in the following manner. Target cells consist of either allogeneic HLA-matched or autologous EBV-transformed B lymphoblastoid cell line that are incubated overnight with the synthetic peptide epitope of the invention at 10

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μM , and labeled with 100 μCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 hour after which they are washed four times with HBSS.

Cytolytic activity is determined in a standard 4 hour, split-well ^{51}Cr release assay using U-bottomed 96 well plates containing 3,000 targets/well. Stimulated PBMC are tested at effector/target (E/T) ratios of 20-50:1 on day 14. Percent cytotoxicity is determined from the formula: $100 \times [(\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})]$. Maximum release is determined by lysis of targets by detergent (2% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release is <25% of maximum release for all experiments.

The results of such an analysis indicate the extent to which HLA-restricted CTL populations have been stimulated by previous exposure to the TAA or TAA vaccine.

The class II restricted HTL responses may also be analyzed. Purified PBMC are cultured in a 96-well flat bottom plate at a density of 1.5×10^5 cells/well and are stimulated with 10 $\mu\text{g}/\text{ml}$ synthetic peptide, whole antigen, or PHA. Cells are routinely plated in replicates of 4-6 wells for each condition. After seven days of culture, the medium is removed and replaced with fresh medium containing 10U/ml IL-2. Two days later, 1 μCi ^3H -thymidine is added to each well and incubation is continued for an additional 18 hours. Cellular DNA is then harvested on glass fiber mats and analyzed for ^3H -thymidine incorporation. Antigen-specific T cell proliferation is calculated as the ratio of ^3H -thymidine incorporation in the presence of antigen divided by the ^3H -thymidine incorporation in the absence of antigen.

Example 18. Induction Of Specific CTL Response In Humans

A human clinical trial for an immunogenic composition comprising CTL and HTL epitopes of the invention is set up as an IND Phase I, dose escalation study. Such a trial is designed, for example, as follows:

A total of about 27 subjects are enrolled and divided into 3 groups:

Group I: 3 subjects are injected with placebo and 6 subjects are injected with 5 μg of peptide composition;

Group II: 3 subjects are injected with placebo and 6 subjects are injected with 50 μg peptide composition;

Group III: 3 subjects are injected with placebo and 6 subjects are injected with 500 μg of peptide composition.

After 4 weeks following the first injection, all subjects receive a booster inoculation at the same dosage. Additional booster inoculations can be administered on the same schedule.

5 The endpoints measured in this study relate to the safety and tolerability of the peptide composition as well as its immunogenicity. Cellular immune responses to the peptide composition are an index of the intrinsic activity of the peptide composition, and can therefore be viewed as a measure of biological efficacy. The following summarize the clinical and laboratory data that relate to safety and efficacy endpoints.

10 Safety: The incidence of adverse events is monitored in the placebo and drug treatment group and assessed in terms of degree and reversibility.

Evaluation of Vaccine Efficacy: For evaluation of vaccine efficacy, subjects are bled before and after injection. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

15 The vaccine is found to be both safe and efficacious.

Example 19. Therapeutic Use in Cancer Patients

20 Evaluation of vaccine compositions are performed to validate the efficacy of the CTL-HTL peptide compositions in cancer patients. The main objectives of the trials are to determine an effective dose and regimen for inducing CTLs in cancer patients, to establish the safety of inducing a CTL and HTL response in these patients, and to see to what extent activation of CTLs improves the clinical picture of cancer patients, as manifested by a reduction in tumor cell numbers. Such a study is designed, for example, as follows:

25 The studies are performed in multiple centers. The trial design is an open-label, uncontrolled, dose escalation protocol wherein the peptide composition is administered as a single dose followed six weeks later by a single booster shot of the same dose. The dosages are 50, 500 and 5,000 micrograms per injection. Drug-associated adverse effects (severity and reversibility) are recorded.

30 There are three patient groupings. The first group is injected with 50 micrograms of the peptide composition and the second and third groups with 500 and 5,000 micrograms of peptide composition, respectively. The patients within each group range in age from 21-65, include both males and females (unless the tumor is sex-specific, e.g., breast or prostate cancer), and represent diverse ethnic backgrounds.

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Example 20. Induction of CTL Responses Using a Prime Boost Protocol

A prime boost protocol similar in its underlying principle to that used to evaluate the efficacy of a DNA vaccine in transgenic mice, which was described in Example 12, may also be used for the administration of the vaccine to humans. Such a vaccine regimen may include an initial administration of, for example, naked DNA followed by a boost using recombinant virus encoding the vaccine, or recombinant protein/polypeptide or a peptide mixture administered in an adjuvant.

For example, the initial immunization may be performed using an expression vector, such as that constructed in Example 11, in the form of naked nucleic acid administered IM (or SC or ID) in the amounts of 0.5-5 mg at multiple sites. The nucleic acid (0.1 to 1000 μ g) can also be administered using a gene gun. Following an incubation period of 3-4 weeks, a booster dose is then administered. The booster can be recombinant fowlpox virus administered at a dose of $5 \cdot 10^7$ to $5 \cdot 10^9$ pfu. An alternative recombinant virus, such as an MVA, canarypox, adenovirus, or adeno-associated virus, can also be used for the booster, or the polypeptidic protein or a mixture of the peptides can be administered. For evaluation of vaccine efficacy, patient blood samples will be obtained before immunization as well as at intervals following administration of the initial vaccine and booster doses of the vaccine. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

Analysis of the results will indicate that a magnitude of response sufficient to achieve protective immunity against cancer is generated.

Example 21. Administration of Vaccine Compositions Using Dendritic Cells

Vaccines comprising peptide epitopes of the invention may be administered using dendritic cells. In this example, the immunogenic peptide epitopes are used to elicit a CTL and/or HTL response *ex vivo*.

Ex vivo CTL or HTL responses to a particular tumor-associated antigen are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptides. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will

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destroy (CTL) or facilitate destruction (HTL) of their specific target cells, *i.e.*, tumor cells.

Alternatively, the peptide-pulsed dendritic cells may be administered to the patient to stimulate a CTL response *in vivo*. In this method, dendritic cells are isolated as described in Example 3. The dendritic cell population is expanded and pulsed with a vaccine comprising peptide CTL and HTL epitopes of the invention. The dendritic cells are infused back into the patient to elicit CTL and HTL responses *in vivo*. The induced CTL and HTL then destroy (CTL) or facilitate destruction (HTL) of the specific target tumor cells that bear the proteins from which the epitopes in the vaccine are derived.

Example 22. Alternative Method of Identifying Motif-Bearing Peptides

Another way of identifying motif-bearing peptides is to elute them from cells bearing defined MHC molecules. For example, EBV transformed B cell lines used for tissue typing, have been extensively characterized to determine which HLA molecules they express. In certain cases these cells express only a single type of HLA molecule. These cells can then be infected with a pathogenic organism or transfected with nucleic acids that express the tumor antigen of interest. Thereafter, peptides produced by endogenous antigen processing of peptides produced consequent to infection (or as a result of transfection) will bind to HLA molecules within the cell and be transported and displayed on the cell surface.

The peptides are then eluted from the HLA molecules by exposure to mild acid conditions and their amino acid sequence determined, *e.g.*, by mass spectral analysis (*e.g.*, Kubo *et al.*, *J. Immunol.* 152:3913, 1994). Because, as disclosed herein, the majority of peptides that bind a particular HLA molecule are motif-bearing, this is an alternative modality for obtaining the motif-bearing peptides correlated with the particular HLA molecule expressed on the cell.

Alternatively, cell lines that do not express any endogenous HLA molecules can be transfected with an expression construct encoding a single HLA allele. These cells may then be used as described, *i.e.*, they may be infected with a pathogenic organism or transfected with nucleic acid encoding an antigen of interest to isolate peptides corresponding to the pathogen or antigen of interest that have been presented on the cell surface. Peptides obtained from such an analysis will bear motif(s) that correspond to binding to the single HLA allele that is expressed in the cell.

As appreciated by one in the art, one can perform a similar analysis on a cell bearing more than one HLA allele and subsequently determine peptides specific for each HLA allele expressed. Moreover, one of skill would also recognize that means other than infection or transfection, such as loading with a protein antigen, can be used to provide a source of antigen to the cell.

The above examples are provided to illustrate the invention but not to limit its scope. For example, the human terminology for the Major Histocompatibility Complex, namely HLA, is used throughout this document. It is to be appreciated that these principles can be extended to other species as well. Thus, other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent application cited herein are hereby incorporated by reference for all purposes.

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TABLE I

SUPERMOTIFS	POSITION	POSITION	POSITION
	2 (Primary Anchor)	3 (Primary Anchor)	C Terminus (Primary Anchor)
A1	T <i>L</i> V <i>M</i> S		F <i>W</i> Y
A2	L <i>I</i> V <i>M</i> A <i>T</i> Q		I <i>V</i> M <i>ATL</i>
A3	V <i>S</i> M <i>A</i> T <i>L</i> I		R K
A24	Y <i>F</i> W <i>I</i> V <i>L</i> M T		F <i>I</i> Y <i>W</i> L M
B7	P		V <i>I</i> L <i>F</i> M <i>W</i> Y A
B27	R H K		F <i>Y</i> L <i>W</i> M <i>IVA</i>
B44	E D		F <i>W</i> Y <i>L</i> I M V A
B58	A T S		F <i>W</i> Y <i>L</i> I V M A
B62	Q <i>L</i> I <i>V</i> M P		F <i>W</i> Y <i>M</i> I <i>V</i> L A
MOTIFS			
A1	T S M		Y
A1		D E A S	Y
A2.1	L <i>M</i> V <i>Q</i> I A T		V <i>L</i> I M A T
A3	L <i>M</i> V <i>IS<i>A</i>T<i>F</i>C<i>G</i>D</i>		K <i>Y</i> R <i>H</i> F A
A11	V <i>T</i> M <i>L</i> I S <i>A</i> G <i>N</i> C <i>D</i> F		K <i>R</i> Y H
A24	Y F W M		F L I W
A*3101	M <i>V</i> T <i>A</i> L I S		R K
A*3301	M <i>V</i> A <i>L</i> F <i>IST</i>		R K
A*6801	A <i>V</i> T <i>M</i> S <i>LI</i>		R K
B*0702	P		L <i>M</i> F <i>W</i> Y <i>A</i> I V
B*3501	P		L <i>M</i> F <i>W</i> Y <i>IVA</i>
B51	P		L <i>I</i> V <i>F</i> W Y A M
B*5301	P		I <i>M</i> F <i>W</i> Y <i>A</i> L V
B*5401	P		A <i>T</i> I <i>V</i> L <i>M</i> F <i>W</i> Y

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE Ia

SUPERMOTIFS	POSITION	POSITION	POSITION
	2 (Primary Anchor)	3 (Primary Anchor)	C Terminus (Primary Anchor)
A1	TLVMS		FWY
A2	<i>VQAT</i>		<i>VLIMAT</i>
A3	<i>VSMATLI</i>		RK
A24	<i>YFWIVLMT</i>		<i>FIYWLM</i>
B7	P		<i>VILFMWYA</i>
B27	RHK		<i>FYLWMIVA</i>
B58	ATS		<i>FWYLVMA</i>
B62	<i>QLIVMP</i>		<i>FWYMIVLA</i>
MOTIFS			
A1	TSM		Y
A1		DEAS	Y
A2.1	<i>VQAT*</i>		<i>VLIMAT</i>
A3.2	LMVISATFCGD		KYRHFA
A11	VTMLISAGNCDF		KRHY
A24	YFW		FLIW

*If 2 is V, or Q, the C-term is not L

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE II

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
<u>SUPERMOTIFS</u>										
A1			1° Anchor TLVMS							1° Anchor FWY
A2			1° Anchor LIVMATQ							1° Anchor LIVMAT
A3	preferred		1° Anchor VSMATLI	YFW (4/5)			YFW (3/5)	YFW (4/5)	P (4/5)	1° Anchor RK
	deleterious	DE (3/5); P (5/5)		DE (4/5)						
A24			1° Anchor YFWIVLM T							1° Anchor FIYWLM
B7	preferred	FWY (5/5) LIVM (3/5)	1° Anchor P	FWY (4/5)					FWY (3/5)	1° Anchor VILFMWYA
	deleterious	DE (3/5); P(5/5); G(4/5); A(3/5); QN (3/5)				DE (3/5)	G (4/5)	QN (4/5)	DE (4/5)	
B27			1° Anchor RHK							1° Anchor FYLHMIYA
B44			1° Anchor ED							1° Anchor FWYLIMVA
B58			1° Anchor ATS							1° Anchor FWYLIYMA
B62			1° Anchor QLIYMP							1° Anchor FWYMIYLA

POSITION								
1	2	3	4	5	6	7	8	C-terminus

POSITION								
1	2	3	4	5	6	7	8	C-terminus
A1 preferred	GFYW	DEA	YFW	P	DEQN	YFW	$\frac{1^{\circ}\text{Anchor}}{Y}$	
9-mer	$\frac{1^{\circ}\text{Anchor}}{STM}$							
deleterious	DE	RHKLIVM P	A	G	A			

MOTIFS

A1 preferred	GRHK	ASTCLIV M	$\frac{1^{\circ}\text{Anchor}}{DE4S}$	GSTC	ASTC	LIVM	DE	$\frac{1^{\circ}\text{Anchor}}{Y}$
9-mer								
deleterious	A	RHKDEPY FW	DE	PQN	RHK	PG	GP	

POSITION

	1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A1 preferred 10-mer	YFW	<u>1°Anchor</u> STM	DEAQN	A	YFWQN		PASTC	GDE	P	<u>1°Anchor</u> Y
deleterious	GP		RHKGLIV M	DE	RHK	QNA	RHKYFW	RHK	A	
A1 preferred 10-mer	YFW	STCLIVM	<u>1°Anchor</u> DEAS	A	YFW		PG	G	YFW	<u>1°Anchor</u> Y
deleterious	RHK	RHKDEPY FW			P	G		PRHK	QN	
A2.1 preferred 9-mer	YFW	<u>1°Anchor</u> LMI/VQAT	YFW	STC	YFW		A	P	<u>1°Anchor</u> VLIMAT	
deleterious	DEP		DERKH			RKH	DERKH			
A2.1 preferred 10-mer	AYFW	<u>1°Anchor</u> LMI/VQAT	LVIM	G		G		FYWL VIM		<u>1°Anchor</u> VLIMAT
deleterious	DEP		DE	RKHA	P		RKH	DERK H	RKH	

POSITION									
	1	2	3	4	5	6	7	8	C-terminus or C-terminus
A3 preferred	RHK	<u>1°Anchor</u> LMVISAT FCGD	YFW	PRHKYFW	A	YFW		P	<u>1°Anchor</u> KYRHFA
deleterious	DEP		DE						
A11 preferred	A	<u>1°Anchor</u> VTLMISA GNCDF	YFW	YFW	A	YFW	YFW	P	<u>1°Anchor</u> KRYH
deleterious	DEP						A	G	
A24 9-mer preferred	YFWRHK	<u>1°Anchor</u> YFWM		STC			YFW	YFW	<u>1°Anchor</u> FLIW
deleterious	DEG		DE	G	QNP	DERHK	G	AQN	
A24 10-mer preferred		<u>1°Anchor</u> YFWM		P	YFWP		P		<u>1°Anchor</u> FLIW
deleterious			GDE	QN	RHK	DE	A	QN	DEA

POSITION									
	1	2	3	4	5	6	7	8	9 or C-terminus
A3101 preferred	RHK	<u>1°Anchor</u> MVTALIS	YFW	P		YFW	YFW	AP	<u>1°Anchor</u> RK
deleterious	DEP		DE		ADE	DE	DE	DE	
A3301 preferred		<u>1°Anchor</u> MVALF/S T	YFW				AYFW		<u>1°Anchor</u> RK
deleterious	GP		DE						
A6801 preferred	YFWSTC	<u>1°Anchor</u> AVTMSLI			YFWLIIV M		YFW	P	<u>1°Anchor</u> RK
deleterious	GP		DEG		RHK			A	
B0702 preferred	RHKFWY	<u>1°Anchor</u> P	RHK		RHK	RHK	RHK	PA	<u>1°Anchor</u> LMFWYIIV
deleterious	DEQNP		DEP	DE	DE	GDE	QN	DE	
B3501 preferred	FWYLIVM	<u>1°Anchor</u> P	FWY				FWY		<u>1°Anchor</u> LMFWYIIV
deleterious	AGP				G	G			

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		POSITION								
		1	2	3	4	5	6	7	8	9 or C-terminus
B51	preferred	LIVMFYW	<u>1°Anchor</u> P	FWY	STC	FWY	FWY	G	FWY	<u>1°Anchor</u> LIVFWYAM
	deleterious	AGPDERHKSTC				DE	G	DEQN	GDE	
B5301	preferred	LIVMFYW	<u>1°Anchor</u> P	FWY	STC	FWY		LIVMFYW	FWY	<u>1°Anchor</u> IMFWYALV
	deleterious	AGPQN					G	RHKQN	DE	
B5401	preferred	FWY	<u>1°Anchor</u> P	FWYLIVM		LIVM		ALIVM	FWYAP	<u>1°Anchor</u> ATVLMFW Y
	deleterious	GPQNDE		GDESTC		RHKDE	DE	QNDGE	DE	

Italicized residues indicate less preferred or "tolerated" residues.
The information in Table II is specific for 9-mers unless otherwise specified.

TABLE III

		POSITION								
		1° anchor 1	2	3	4	5	1° anchor 6	7	8	9
MOTIFS										
DR4 preferred	deleterious	FMVLIW	M	T	W	I	VSTCPALM	MH		MH
								R		WDE
DR1 preferred	deleterious	MFLIWW	C	CH	PAMQ	CWD	VMATSP/LC	M	GDE	D
										AVM
DR7 preferred	deleterious	MFLIWW	M	W	A		IVMSACTPL	M		IV
			C		G			GRD		G
DR Supermotif		MFLIWW					VMSTACP/LI			
DR3 MOTIFS		1° anchor 1	2	3	1° anchor 4	5	1° anchor 6			
motif a preferred		LIVMFY			D					
motif b preferred		LIVMFAY			DNQEST		KRH			

Italicized residues indicate less preferred or "tolerated" residues.

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TABLE III

MOTIFS	POSITION					
	1° anchor 1	2	3	4	5	1° anchor 6
DR4 preferred	FMYLIVW	M	T		I	VSTCPALIM
deleterious				W		R
DR1 preferred	MFLIVWY			PAMQ		M
deleterious		C	CH	FD	CWD	GDE
DR7 preferred	MFLIVWY	M	W	A		M
deleterious		C		G		GRD
DR Supermotif	MFLIVWY					
DR3 MOTIFS	1° anchor 1	2	3	1° anchor 4	5	1° anchor 6
motif a preferred	LIVMFY			D		
motif b preferred	LIVMFAY			DNQEST		KRH
						VMSTACPLI
						VMATSP LIC
						IVMSACTPL
						GRD
						N
						G
						IV
						G
						AVM
						D
						MH
						WDE

Italicized residues indicate less preferred or "tolerated" residues.

Chs 931
93
Table IV. HLA Class I Standard Peptide Binding Affinity.

ALLELE	STANDARD PEPTIDE	SEQUENCE	STANDARD BINDING AFFINITY (nM)
A*0101	944.02	YLEPAIAKY	25
A*0201	941.01	FLPSDYFPSV	5.0
A*0202	941.01	FLPSDYFPSV	4.3
A*0203	941.01	FLPSDYFPSV	10
A*0205	941.01	FLPSDYFPSV	4.3
A*0206	941.01	FLPSDYFPSV	3.7
A*0207	941.01	FLPSDYFPSV	23
A*6802	1072.34	YVIKVSARV	8.0
A*0301	941.12	KVFPYALINK	11
A*1101	940.06	AVDLYHFLK	6.0
A*3101	941.12	KVFPYALINK	18
A*3301	1083.02	STLPETYVRR	29
A*6801	941.12	KVFPYALINK	8.0
A*2402	979.02	AYIDNYNKF	12
B*0702	1075.23	APRTLVL	5.5
B*3501	1021.05	FPFKYAAAF	7.2
B51	1021.05	FPFKYAAAF	5.5
B*5301	1021.05	FPFKYAAAF	9.3
B*5401	1021.05	FPFKYAAAF	10

Chasyl
ay
~~Table V. HLA Class II Standard Peptide Binding Affinity.~~

Allele	Nomenclature	Standard Peptide	Sequence	Binding Affinity (nM)
DRB1*0101	DR1	515.01	PKYVKQNTLKLAT	5.0
DRB1*0301	DR3	829.02	YKTIAFDEEARR	300
DRB1*0401	DR4w4	515.01	PKYVKQNTLKLAT	45
DRB1*0404	DR4w14	717.01	YARFQSQTTLKQKT	50
DRB1*0405	DR4w15	717.01	YARFQSQTTLKQKT	38
DRB1*0701	DR7	553.01	QYIKANSKFIGITE	25
DRB1*0802	DR8w2	553.01	QYIKANSKFIGITE	49
DRB1*0803	DR8w3	553.01	QYIKANSKFIGITE	1600
DRB1*0901	DR9	553.01	QYIKANSKFIGITE	75
DRB1*1101	DR5w11	553.01	QYIKANSKFIGITE	20
DRB1*1201	DR5w12	1200.05	EALIHQLKINPYVLS	298
DRB1*1302	DR6w19	650.22	QYIKANAKFIGITE	3.5
DRB1*1501	DR2w2 β 1	507.02	GRTQDENPVVHFFKNIV TPRTPPP	9.1
DRB3*0101	DR52a	511	NGQIGNDPNRDIL	470
DRB4*0101	DRw53	717.01	YARFQSQTTLKQKT	58
DRB5*0101	DR2w2 β 2	553.01	QYIKANSKFIGITE	20

~~The "Nomenclature" column lists the allelic designations used in Tables XIX and XX.~~

Table VI

HLA supertype	Allele-specific HLA-supertype members	
	Verified ^a	Predicted ^b
A1	A*0101, A*2501, A*2601, A*2602, A*3201	A*0102, A*2604, A*3601, A*4301, A*8001
A2	A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, A*6901	A*0208, A*0210, A*0211, A*0212, A*0213
A3	A*0301, A*1101, A*3101, A*3301, A*6801	A*0302, A*1102, A*2603, A*3302, A*3303, A*3401, A*3402, A*6601, A*6602, A*7401
A24	A*2301, A*2402, A*3001	A*2403, A*2404, A*3002, A*3003
B7	B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, B*7801	B*1511, B*4201, B*5901
B27	B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, B*7301	B*2701, B*2707, B*2708, B*3802, B*3903, B*3904, B*3905, B*4801, B*4802, B*1510, B*1518, B*1503
B44	B*1801, B*1802, B*3701, B*4402, B*4403, B*4404, B*4001, B*4002, B*4006	B*4101, B*4501, B*4701, B*4901, B*5001
B58	B*5701, B*5702, B*5801, B*5802, B*1516, B*1517	
B62	B*1501, B*1502, B*1513, B*5201	B*1301, B*1302, B*1504, B*1505, B*1506, B*1507, B*1515, B*1520, B*1521, B*1512, B*1514, B*1510

- Verified alleles included alleles whose specificity has been determined by pool sequencing analysis, peptide binding assays, or by analysis of the sequences of CTL epitopes.
- Predicted alleles are alleles whose specificity is predicted on the basis of B and F pocket structure to overlap with the supertype specificity.

Table VII
p53 A01 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0101	SEQ ID NO.
CTTHIYNY	229	8	0.0460	1
CTYSPALNKM	124	11		2
EVGSDCTTHY	224	11		3
FTLQIRGRERF	328	11		4
GSDCTTHY	226	9	29.5000	5
GSDCTTHIYNY	226	11	0.3700	6
GSYGRFLGF	105	9		7
GTAKSVTCTY	117	10	0.3100	8
GTRVRAMAIY	154	10	0.0027	9
KTCPVOLW	139	8		10
KTYQGSYGF	101	9		11
LMLSPDDIEQW	43	11		12
LSPDDIEQW	45	9		13
LSPDDIEQWF	45	10		14
LSQETFSDLW	14	10		15
LSSVPSQKTY	93	11	-0.0012	16
MLSPDDIEQW	44	10		17
MLSPDDIEQWF	44	11		18
NLLGRNSF	263	8		19
NTRISVVVPY	210	11	0.0022	20
PLSQETFSDLW	13	11	0.0140	21
PSQKTYQGSY	98	10		22
QIRGRERF	331	8		23
QIRGRERFEMF	331	11		24
QLAKTCPVOLW	136	11		25
QTSRIKKLMF	375	11		26
RVEGNLRVEY	196	10	0.0220	27
RVEYDDRNTF	202	11		28
RVRAMAIY	156	8		29
SSGNLLGRNSF	260	11		30
SSSVPSQKTY	94	10	0.0010	31
SSVPSQKTY	95	9	0.0014	32
STSRHKLMF	376	10		33
SVPTLSQETF	9	11		34
SVPSQKTY	96	8		35
TLOIRGRERF	329	10		36
TSRIKKLMF	377	9		37
YDDRNTF	205	8		38
YSPALNKM	126	9		39

Table VIII
p53 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
AAPAPAPSWPL	83	11	0.0001					40
AAPVAPAPA	69	8	0.0007					41
AAPVAPAPAPA	69	10	0.0003				0.0017	42
AAPVAPAPAAA	69	11	0.0001					43
AAPTAPAPA	78	9	0.0005					44
AAPTAAPAPA	78	11	0.0001					45
AIYKQSQIM	161	9	0.0001					46
AIYKQSQIMT	161	10	0.0001					47
ALELKDAQA	347	9	0.0024					48
ALNKMFCQL	129	9	0.0013					49
ALNKMFCQLA	129	10	0.0051					50
AMAIYKQSQIM	159	11	0.0003				-0.0003	51
CACGDRDRKT	275	10	0.0001					52
CMGGMNRRPI	242	10	0.0001	0.0030	0.0730	0.0016		53
CMGGMNRRPIL	242	11	0.0001					54
COLAKTCPV	135	9	0.0240					55
COLAKTCVQL	135	11	0.0002	0.1000	0.0700	0.0410	-0.0001	56
CTTIHYNM	229	9	0.0180					57
CTYSPALNKM	124	10	0.0001	0.0150	0.0039	0.0066	0.0440	58
DLMLSPDDI	42	9	0.0001					59
DLWKLPPENV	21	11	0.0001					60
EAAPPVAPA	68	9	0.0002					61
EAAPPVAPAPA	68	11	0.0001					62
EALDKDA	346	8	-0.0002					63
EALDKDAQA	346	10	0.0001					64
EAPRMPEA	62	8	0.0001					65
EAPRMPEAA	62	9	0.0001					66
ELNEALEL	343	8	-0.0002					67
ELNEALELKDA	343	11	-0.0001					68
ELPPGSTKRA	298	10	0.0001					69
ELPPGSTKRAL	298	11	0.0001					70
EMFRELNEA	339	9	0.0006					71
EMFRELNEAL	339	10	0.0002					72
ETFSDLWKL	17	9	0.0001					73
ETFSDLWKLL	17	10	0.0001					74
EVGSDCTT	224	8	0.0001					75
EVGSDCTTI	224	9	0.0001					76
FLHSGTAKSV	113	10	0.0050					77
FLHSGTAKSVT	113	11	0.0010					78
FTEDGPDEA	54	10	0.0002					79
GLAPPOHIL	187	8	-0.0002					80
GLAPPOHILI	187	9	0.0004					81
GLAPPQHURV	187	11	0.1400					82
GMNRRPIL	245	8	-0.0002					83
GMNRRPILT	245	9	0.0001					84
GMNRRPILTI	245	10	0.0001					85
GMNRRPILTII	245	11	0.0002					86
GQSTRIKKL	374	10	-0.0001					87
GQSTRIKKLM	374	11	-0.0001					88
GTAQSVTCT	117	9	0.0001					89

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Table VIII

p53 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
GTRVRAMA	154	8	0.0001					90
GTRVRAMAI	154	9						91
HLIRVEGNL	193	9	0.0003					92
HLIRVEGNLRV	193	11	0.0130	0.0031	0.1200	0.0031	0.0045	93
HLKSKGGST	368	10	0.0001					94
ITLEDSSGNL	254	11	0.0001					95
ITLEDSSGNL	255	10	0.0001					96
ITLEDSSGNL	255	11	0.0027					97
KLLPENNV	24	8	0.0005					98
KLLPENNV	24	9	0.0058					99
KMFCQLAKT	132	9	0.0099	0.1000	0.5100	0.0400	-0.0002	100
KSQJIMTEV	164	9	0.0100	0.0330	0.0590	0.0130	-0.0001	101
KSQJIMTEV	164	10	0.0009					102
KTCPVQLWV	139	9	0.0035	0.0048	0.0040	0.0090	-0.0002	103
KTYQSGYGR	101	11	0.0017	0.0027	0.0009	0.0051	0.0026	104
LAKTCVQL	137	9	0.0001					105
LAKTCVQLWV	137	11	0.0003					106
LAPQILI	188	8	-0.0002					107
LAPQILIRV	188	10	0.0018	0.0025	0.0024	0.0020	-0.0002	108
LIRVEGNL	194	8	-0.0002					109
LIRVEGNLRV	194	10	0.0001					110
LLGRNSFEV	264	9	0.0140					111
LLGRNSFEVRV	264	11	0.0019					112
LLPENNV	25	8	0.0012					113
LLPENNVLSPL	25	11	0.0001					114
LMLSPDI	43	8	0.0001					115
LQIRGRFEM	330	11	-0.0001					116
MAIKKQSQHM	160	10	0.0001					117
MAIKKQSQHMT	160	11	0.0001					118
NLLGRNSFEV	263	10	0.0130					119
NTRISVV	210	8	0.0001					120
NTRISVVV	210	9	0.0001					121
NVLSPLPSQA	30	10	0.0001					122
NVLSPLPSQAM	30	11	0.0004					123
PAAITPA	77	8	0.0001					124
PAAITPAAPA	77	10	0.0001					125
PALNKMFCQL	128	10	0.0001					126
PALNKMFCQLA	128	11	0.0001					127
PAPAAPTA	75	9	0.0001					128
PAPAAPTAA	75	10	0.0001					129
PAPAPSWPL	85	9	0.0001					130
PAPSWPLSSV	87	11	0.0001					131
PILHTL	250	8	0.0010					132
PLDGEYFT	322	8	-0.0002					133
PLDGEYFTL	322	9	0.0001					134
PLDGEYFTLQI	322	11	0.0001					135
PLPSQAMDDL	34	10	0.0001					136
PLPSQAMDDL	34	11	0.0001					137
PLSQETESDL	13	10	0.0001					138
PLSSVPSQKT	92	11	0.0001					139

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Table VIII
p53 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
PQILIRVEGNL	191	11	-0.0001					140
PQPKKKPL	316	8	-0.0001					141
POSDPSVEPPL	4	11	-0.0001					142
PTTAAAPAA	80	9	0.0002					143
PVAPAPAA	72	8	0.0001					144
PVAPAPAAFT	72	10	0.0001					145
PVOLWVDST	142	9	0.0006					146
QAGKEPGGSR	354	11	0.0001					147
QAMDDLML	38	8	0.0001					148
QIRGRERFEM	331	10	0.0001					149
QLAKTCFV	136	8	0.0075					150
QLAKTCVQL	136	10	0.0001					151
RLGFLHSGT	110	9	0.0003					152
RLGFLHSGTA	110	10	0.0001					153
RMPEAAPV	65	9	0.1200					154
RMPEAAPVA	65	10	0.0580	0.0230	0.6900	0.0100	0.0025	155
RVEGNLRLV	196	8	0.0001					156
RVEGNLRLVEYL	196	11	0.0001					157
RVEYLDDRNT	202	10	0.0001					158
SOAMDDLML	37	8	-0.0001					159
SOAMDDLML	37	9	0.0002					160
SQETESDL	15	8	-0.0001					161
SQETESDLWKL	15	11	-0.0001					162
SOIMTEVV	166	8	-0.0001					163
STKRALNNNT	303	10	0.0001					164
STPPPGTRV	149	9	0.0001					165
STPPPGTRVRA	149	11	0.0001					166
STSRIKKL	376	8	0.0001					167
STSRIKKLM	376	9	0.0001					168
SVEPPLSOET	9	10	0.0001					169
SVTCTYSPA	121	9	0.0002					170
SVTCTYSPAL	121	10	0.0001					171
SVVVPYEPPEV	215	11	0.0001					172
TAKSVTCT	118	8	0.0001					173
TLEDSSGNL	256	9	0.0003					174
TLEDSSGNLL	256	10	0.0009					175
TTIHNYM	230	8	0.0001					176
VAPAPAAFT	73	9	0.0002					177
VAPAPAAPTA	73	11	0.0001					178
VLSPLPSOA	31	9	0.0001					179
VLSPLPSOAM	31	10	0.0001					180
VOLWVDST	143	8	0.0001					181
VTCTYSPA	122	8	0.0001					182
VTCTYSPAL	122	9	0.0009					183
VVPYEPPEV	217	9	0.0008					184
VVPYEPPEV	216	10	0.0026	0.0042	0.0240	0.0038	-0.0003	185
WVDSTPPPGT	146	10	0.0002					186
YMCNSSCM	236	8	0.0007					187
YMCNSSCMGM	236	11	0.0099					188
YQGSYGRL	103	9	0.0025					189

Table IX
p53 A03 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801	SEQ ID NO.
ALELKDAQAGK	347	11	0.0012	0.0005				190
ALNKMFCOLAK	129	11	0.4400	0.0420	0.0190	-0.0013	-0.0001	191
CACPGRDR	275	8	-0.0001	-0.0001				192
CACPGRRR	275	9	0.0014	0.0003				193
CMGGMNRR	242	8	0.0003	0.0006				194
CTYSPALNK	124	9	0.4600	1.1000	0.0120	0.0560	0.2200	195
DSSGNLGR	259	9	0.0014	0.0001				196
DSTPPGTR	148	9	0.0014	0.0001				197
DSTPPGTRVR	148	11	-0.0009	-0.0002				198
ELKDAQAGK	349	9	0.0005	0.0001	0.0002	0.0066	0.0130	199
ELNEALELK	343	9	0.0220	0.0052	0.0002	0.0290	0.0810	200
ELPPGSTK	298	8	-0.0004	-0.0003				201
ELPPGSTKR	298	9	0.0002	0.0005				202
ETFSDLWK	17	8	-0.0001	0.0050				203
EVRVCACFGR	271	10	0.0002	0.0001				204
EVVRRCPHIER	171	11	0.0017	-0.0002				205
FLHSGTAK	113	8	0.0130	0.0005				206
FTLQIRGR	328	8	-0.0009	-0.0001				207
FTLQIRGRER	328	10	0.0006	0.0006				208
GLAPQHLIR	187	10	0.0130	0.0006				209
GSAHSHILK	361	10	0.0003	0.0002				210
GTRVRAMAIYK	134	11	1.1000	0.3300	1.1000	0.0014	0.0150	211
HLIRVEGNLR	193	10	0.0002	0.0002				212
HMTVEVVR	168	8	0.0046	0.0003				213
HSHILKSK	365	8	-0.0001	0.0005				214
HSHILKSKK	365	9	0.0014	0.0008				215
KMFCOLAK	132	8	0.3800	0.3600	0.0510	0.0011	0.0110	216
KSKKGQSTR	370	10	0.0240	0.0002				217
KTYQGSYGFR	101	10	2.6000	0.8800				218
LAPQHLIR	188	9	0.0014	0.0001				219
LIRVEGNLR	194	9	0.0005	0.0005				220
LLGRNSFEVR	264	10	0.0002	0.0001				221
LSQETFSDLWK	14	11	-0.0009	0.0470	0.0007	-0.0013	0.0018	222
LSSSVTSQK	93	9	0.0014	0.0028				223
NLLGRNSFEVR	263	11	-0.0009	-0.0002				224
NLRVEYLDLR	200	10	0.0002	0.0001				225
NSSCMGGMNR	239	10	0.0001	0.0320				226
NSSCMGGMNRR	239	11	0.0012	0.0015	0.0002	0.0040	0.0430	227
NTSSSPQPK	311	9	0.0009	0.0950				228
NTSSSPQPKK	311	10	0.0035	0.0540				229
NTSSSPQPKKK	311	11	-0.0009	-0.0002				230
PLSSSVTSQK	92	10	0.0021	0.0002				231
QAGKEPGSR	354	10	0.0001	0.0002				232
QSQHMTVEVR	165	10	0.0014	0.0002				233
QSQHMTVEVRR	165	11	-0.0009	-0.0002				234
QSTRHKK	375	8	0.0004	0.0004				235
RAHSHILK	363	8	0.5300	0.0071	-0.0004	-0.0009	0.0009	236
RAHSHILKSK	363	10	0.0001	0.0002				237
RAHSHILKSKK	363	11	0.0270	0.0038	-0.0006	-0.0013	0.0009	238
RLGLISGTAK	110	11	0.0430	0.0001	-0.0006	-0.0013	-0.0001	239

Table IX
p53 A03 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801	SEQ ID NO.
RTEENLR	283	8	-0.0001	-0.0001	0.0002	0.0006	0.0001	240
RTEENLRK	283	9	0.0015	0.0910				241
RTEENLRKK	283	10	3.3000	0.0080	0.1700	0.1500	0.0140	242
RVCACPR	273	8	0.3500	0.0490				243
RVCACPRDR	273	10	0.0140	0.0110				244
RVCACPRDRR	273	11	0.0290	0.0290	0.0520	-0.0013	0.0120	245
RVEYDDR	202	8	-0.0004	-0.0003				246
RVRAMAIYK	156	9	1.5000	0.7300	3.7000	0.0063	0.0030	247
SSCMGGMNR	240	9	0.0200	1.4000				248
SSCMGGMNRR	240	10	0.0001	0.0860				249
SSGNLLGR	260	8	-0.0005	0.0017				250
SSILKSKK	366	8	0.0005	0.0026				251
SSFPQKK	314	8	-0.0001	-0.0001				252
SSSPQPKK	313	8	-0.0001	0.0013				253
SSSPQPKK	313	9	0.0014	0.0006				254
SSVPQK	94	8	0.0005	0.0010				255
STPPGTR	149	8	-0.0001	-0.0001				256
STPPGTRV	149	10	0.0002	0.0006				257
STSRHKKLMFK	376	11	0.3100	0.1300	0.0610	-0.0013	0.0150	258
TLOIRGR	329	9	0.0002	0.0001				259
TSRHKKLMFK	377	10	0.0500	0.0052				260
TSSSPQK	312	8	-0.0001	0.0019				261
TSSSPQKK	312	9	0.0014	0.0001				262
TSSSPQPKK	312	10	0.0001	0.0002				263
VTCTYSPALNK	122	11	0.0700	0.1200	0.0101	-0.0013	0.0068	264
VVRCPPIIER	172	10	0.0900	0.0017				265
YVDSTPPGTR	146	11	-0.0009	-0.0002				266
YLDDRNTFR	205	9	0.0006	0.0005				267

Table X
p53 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
AIYKOSQIM	161	9		268
ALNKMFCOL	129	9		269
AMAIYKOSQIM	159	11		270
CMGGMNRRIPI	242	10		271
CTTHIYNY	229	8		272
CTTHIYNYM	229	9		273
CTYSPALNKM	124	10		274
CTYSPALNKM	124	11		275
DLMLSPDDI	42	9		276
ELNEALEL	343	8		277
ELPGSTKRAL	298	11		278
EMFRELNEAL	339	10		279
ETFSDLWKL	17	9		280
ETFSDLWKL	17	10		281
EVGSDCTTI	224	9		282
EVGSDCTTHIY	224	11		283
EYLDIDRNTF	204	9	0.0010	284
FTLOIRGRERF	328	11		285
GLATPOHL	187	8		286
GLATPOHLI	187	9		287
GMNRRPIL	245	8		288
GMNRRPILTI	245	10		289
GTAKSVTCTY	117	11		290
GTRVRAMAI	154	9	0.0001	291
GTRVRAMAIY	154	10		292
HLIRVEGNL	193	9		293
IYNYMCMNSSCM	233	11		294
ITLEDSSGNL	254	11		295
ITLEDSSGNL	255	10		296
ITLEDSSGNL	255	11		297
IYKOSQIM	162	8		298
KLLPENNVL	24	9		299
KTCPVQLW	139	8		300
KTYQGSYGF	101	9		301
KTYQGSYGFRL	101	11		302
LIRVEGNL	194	8		303
LLPENNVL	25	8		304
LLPENNVLSP	25	11		305
LMLSPDDI	43	8		306
LMLSPDDIEQW	43	11	0.0023	307
LWKLLENVL	22	11	-0.0003	308
MFRELNEAL	340	9	0.0001	309
MLSPDDIEQW	44	10		310
MLSPDDIEQWF	44	11		311
NLLGRNSF	263	8		312
NTERISVVVPY	210	11		313
NVLSPLFSQAM	30	11		314
				315
				316
				317

660T2T " 262E5460

Table X

p53 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
NYMCNSSCM	235	9		318
PILTIITL	250	8		319
PLDGEYFTL	322	9		320
PLDGEYFTLQI	322	11		321
PLFSQAMDDL	34	10		322
PLFSQAMDDLDM	34	11		323
PLSQETFSPL	13	10		324
PLSQETFSDLW	13	11		325
QIRGRERF	331	8		326
QIRGRERFEM	331	10		327
QIRGRERFEMF	331	11		328
QLAKTCTVQL	136	10		329
QLAKTCTVQLW	136	11		330
RFEMFREL	337	8	-0.0004	331
RVEGNLRVEY	196	10		332
RVEGNLRVEYL	196	11		333
RVEYDDRNTF	202	11		334
RVRAMAIY	156	8		335
STSRHKKL	376	8		336
STSRHKKLM	376	9		337
STSRHKKLMF	376	10		338
SVEPPLSQETF	9	11		339
SVFSOKTY	96	8		340
SVTCTYSPAL	121	10		341
SYGFRLGFL	106	8	0.0280	342
SYGFRLGFL	106	9	0.0200	343
TERIISVVVPY	211	10		344
TFSDLWKL	18	8	0.0016	345
TFSDLWKL	18	9	0.0010	346
TLEDSSGNL	256	9		347
TLEDSSGNL	256	10		348
TLOIRGREKF	230	8		349
TTIHYNM	230	8		350
TYQGSYGF	102	8	0.1100	351
TYQGSYGFRL	102	10	0.1200	352
TYSPALNKM	125	9		353
TYSPALNKM	125	10		354
VLSPLFSQAM	31	10	5.1000	355
VTCTYSPAL	122	9		356
YLDNRNTF	205	8		357
YMCNSSCM	236	8		358
YMCNSSCMGGM	236	11		359

Table XI
p51 B07 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	B*0702	SEQ ID NO.
APAAPTPA	76	8	0.0036	360
APAAPTPAA	76	9	0.3000	361
APAAPTAAPA	76	11	0.3900	362
APAAAPTTPA	74	10	0.0190	363
APAAAPTTPAA	74	11	0.0390	364
APAPAPSW	84	8	0.0062	365
APAPAPSWPL	84	10	0.5700	366
APAPSWPL	86	8	0.0540	367
APQHLIRV	189	9	0.0005	368
APPVAPAPA	70	9	0.0028	369
APPVAPAPAA	70	10	0.0098	370
APRMPEAA	63	8	0.0170	371
APRMPEAAAPPV	63	11	0.4500	372
APSWPLSSSV	88	10	0.0230	373
APTAAAPA	79	8	0.0013	374
APTAAAPAPA	79	10	0.0013	375
DPGPDAPRM	57	10	-0.0003	376
DPVSEPTL	7	8	-0.0002	377
EPPLSQETF	11	9	-0.0003	378
EPQSDPSV	3	8	-0.0002	379
GPDEAPRM	59	8	0.0004	380
GPDEAPRMPEA	59	11	0.0008	381
KPLDGEYF	321	8	-0.0002	382
KPLDGEYFTL	321	10	0.0055	383
LPENNVLSP	26	10	0.0070	384
LPYGSTKRA	299	9	0.1300	385
LPYGSTKRAL	299	10	0.0038	386
LPSOAMDDL	35	9	-0.0003	387
LPSOAMDDLML	35	10	0.0001	388
LPSOAMDDLML	35	11	0.0028	389
MPEAAAPPV	66	8	-0.0003	390
MPEAAAPPVA	66	9	0.0006	391
MPEAAAPPVAPA	66	11	0.0001	392
PPEVGSDDCTI	222	11	-0.0002	393
PPGSKRA	300	8	0.0005	394
PPGSKRAL	300	9	-0.0002	395
PPGTRVRA	152	8	-0.0002	396
PPGTRVRAM	152	9	-0.0003	397
PPGTRVRAMA	152	10	-0.0003	398
PPGTRVRAMAI	152	11	0.0001	399
PPLSQETF	12	8	-0.0002	400
PPLSQETFSDL	12	11	0.0001	401
PPGTRVRA	151	9	-0.0003	402
PPGTRVRAM	151	10	-0.0003	403
PPGTRVRAMA	151	11	-0.0001	404
PPQHLIRV	190	8	-0.0002	405
PPVAPAPA	71	8	-0.0002	406
PPVAPAPAA	71	9	-0.0003	407
QPKKKPLDGEY	317	11	-0.0004	408
RPILTIITL	249	9	0.3000	409

Table XI
p53 B07 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	B*0702	SEQ ID NO.
SPALNKMIF	127	8	0.0130	410
SPALNKMFCQL	127	11	0.0310	411
SPDDIEQW	46	8	-0.0002	412
SPDDIEQWF	46	9	-0.0003	413
SPLPSQAM	33	8	0.0044	414
SPLPSQAMDDL	33	11	0.0004	415
SPQPKKKPL	315	9	0.1700	416
TPAAPAPA	81	8	0.0041	417
TPAAPAPAPSW	81	11	0.0009	418
TPPPGTRV	150	8	-0.0002	419
TPPPGTRVRA	150	10	-0.0003	420
TPPPGTRVRAM	150	11	-0.0004	421
VPSQKTYQGSY	97	11	-0.0004	422
VYVEPEV	218	8	-0.0002	423

Table XII
p53 B27 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AKSVTCTY	119	8	424
AKTCPVOL	138	8	425
AKTCPVOLW	138	9	426
DRRTEENL	281	9	427
ERCSDSGL	180	9	428
ERFEMFREL	336	9	429
FRELNEAL	341	8	430
FRELNEALEL	341	10	431
FRIISVVVY	212	9	432
GRDRRTEENL	279	11	433
GRERFEMF	334	8	434
GRERFEMFREL	334	11	435
HHERCSDSGL	178	11	436
IRGRERFEM	332	9	437
IRGRERFEMF	332	10	438
IRVEGNLRVEY	195	11	439
KKGEPHIEL	291	9	440
KKKPLDGEY	319	9	441
KKKPLDGEYF	319	10	442
KKPLDGEY	320	8	443
KKPLDGEYF	320	9	444
KKPLDGEYFTL	320	11	445
LKKGEPHIEL	289	11	446
NRRLTII	247	8	447
NRRLTII	247	9	448
NRRLTII	247	11	449
PKKPLDGEY	318	10	450
PKKPLDGEYF	318	11	451
OHLRVFGNL	192	10	452
OKTYQGSY	100	8	453
OKTYQGSYGF	100	10	454
RHSVVVY	213	8	455
RKKGEPHIEL	290	10	456
RRPILTI	248	8	457
RRPILTI	248	10	458
RRTEENL	282	8	459
SRAHSHIL	362	8	460
SRIKKLMF	378	8	461
TRVRAMAI	155	8	462
TRVRAMAIY	155	9	463
WKLPPENNVL	23	10	464

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Table XIII
p53 B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AAPAPAPSW	83	9	465
AAPAPAPSWPL	83	11	466
CTTHIYNY	229	8	467
CTTHIYNYM	229	9	468
CTYSPALNKM	124	10	469
CTYSPALNKMFM	124	11	470
DSDDLAPQIHL	184	11	471
DSTPPGTRV	148	10	472
ETFSDLWKL	17	9	473
ETFSDLWKLL	17	10	474
FSDLWKLL	19	8	475
FTLQIRGRERF	328	11	476
GSDCTTHY	226	9	477
GSDCTTHIYNY	226	11	478
GSAHSSHL	361	9	479
GSYGFRLGF	105	9	480
GSYGFRLGFL	105	10	481
GTAKSVTCTY	117	10	482
GTRVRAMAI	154	9	483
GTRVRAMIV	154	10	484
HSGTAKSV	115	8	485
ITLEDSSGNL	255	10	486
ITLEDSSGNLL	255	11	487
KSVTCTYSPAL	120	11	488
KTCPVLW	139	8	489
KTCPVLWV	139	9	490
KTYQGSYGF	101	9	491
KTYQGSYGFRL	101	11	492
LAKTCPVL	137	9	493
LAKTCPVLW	137	10	494
LAKTCPVLWV	137	11	495
LAPQHLI	188	8	496
LAPQHLIRV	188	10	497
LSPDDIEQW	45	9	498
LSPDDIEQWF	45	10	499
LSPLFSQAM	32	9	500
LSQETESDL	14	9	501
LSQETESDLW	14	10	502
LSSSVPSQKTY	93	11	503
MAIKOSQHIM	160	10	504
NSSCMGGM	239	8	505
NTERHSVV	210	8	506
NTERHSVVV	210	9	507
NTERHSVVVVPY	210	11	508
PAAPAPAPSW	82	10	509
PALNKMFCQL	128	10	510
PAPAPAPSWPL	85	9	511
PAPSWPLSSSV	87	11	512
PSQAMDDL	36	8	513
PSQAMDDLDM	36	9	514

Table XIII
p53 B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
PSOAMDDLML	36	10	515
PSQKTYOGSY	98	10	516
PSWPLSSV	89	9	517
OAMDDLML	38	8	518
QSDPSVEPPL	5	10	519
QSOIMTEV	165	8	520
QSOIMTEVV	165	9	521
QSTSRIKKL	375	9	522
QSTSRIKKLM	375	10	523
QSTSRIKKLMF	375	11	524
SSGNLLGRNSF	260	11	525
SSPQPKKKPL	314	10	526
SSSPQPKKKPL	313	11	527
SSSVPSOKTY	94	10	528
SSVPSOKTY	95	9	529
STPPPGTRV	149	9	530
STRIKKL	376	8	531
STRIKKLM	376	9	532
STRIKKLMF	376	10	533
TAKSVTCTY	118	9	534
TSRIKKLM	377	8	535
TSRIKKLMF	377	9	536
TTHVNYM	230	8	537
VTCTYSPAL	122	9	538
YSPALNKM	126	8	539
YSPALNKMf	126	9	540

650T2T 16285450

Table XIV
p53 B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AIYKSOHIM	161	9	541
AMAIYKSOQHIM	159	11	542
APAPAPSW	84	8	543
APPOHLIRV	189	9	544
APRMPEAAPV	63	11	545
APSWPLSSSV	88	10	546
CMGGMNRRPI	242	10	547
COLAKTCPV	135	9	548
DLMLSPDDI	42	9	549
DLWKLLPENN	21	11	550
DPGPDAPRM	57	10	551
EPLSQETF	11	9	552
EPOSPPSV	3	8	553
EVGSDCTTI	224	9	554
EVGSDCTTHY	224	11	555
FLHSGTAKSV	113	10	556
GLAPQHLI	187	9	557
GLAPPQHILRV	187	11	558
GMNRRPILTI	245	10	559
GMNRRPILTI	245	11	560
GPDEAPRM	59	8	561
GQSTRHKMLM	374	11	562
HLIRVEGNLRV	193	11	563
KLLPENNV	24	8	564
KPLDGEYF	321	8	565
KSQHIMTEV	164	9	566
KSQHIMTEV	164	10	567
LIRVEGNLRV	194	10	568
LLGRNSFEV	264	9	569
LLGRNSFEVRV	264	11	570
LMLSPDDI	43	8	571
LMLSPDDIEQW	43	11	572
LPSQAMDDL	35	10	573
LOIRGRERF	330	9	574
LOIRGRERFEM	330	11	575
MLSPDDIEQW	44	10	576
MLSPDDIEQWF	44	11	577
MPEAAPV	66	8	578
NLLGRNSF	263	8	579
NLLGRNSFEV	263	10	580
NVLSPSQAM	30	11	581
PLDGEYFTLOI	322	11	582
PLPSQAMDDL	34	11	583
PLSQETFSDLW	13	11	584
PPEVGSDDCTI	222	11	585
PPGTRVRAM	152	9	586
PPGTRVRAMAI	152	11	587
PPLSQETF	12	8	588
PPPGTRVRAM	151	10	589
PPQHILRV	190	8	590

Table XIV
p53 B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
QIRGRERF	331	8	591
QIRGRERFEM	331	10	592
QIRGRERFEMF	331	11	593
QLAKTCPV	136	8	594
QLAKTCVQLW	136	11	595
QPKKKPLDGEY	317	11	596
RMPEAAPV	65	9	597
RVEGNLRV	196	8	598
RVEGNLRVEY	196	10	599
RVEYLDNRTE	202	11	600
RVRAMAY	156	8	601
SPALNKM	127	8	602
SPDIEQW	46	8	603
SPDDIEQWF	46	9	604
SPLFSQAM	33	8	605
SOAMDDL	37	8	606
SQETFDLW	15	9	607
SQHMTVV	166	8	608
SOKTYQGSY	99	9	609
SOKTYQGSYGF	99	11	610
SVEPTLSQET	9	11	611
SVFSQKT	96	8	612
SVVVPYEPPEV	215	11	613
TLQIRGRERF	329	10	614
TPAAPAPAFSW	81	11	615
TPPGTRV	150	8	616
TPPGTRVRAM	150	11	617
VLSPLFSQAM	31	10	618
VPSOKTYQGSY	97	11	619
VPEPPEV	218	8	620
VVPYEPPEV	217	9	621
VVPYEPPEV	216	10	622
YLDNRTE	205	8	623
YMCNSSCM	236	8	624
YMCNSSCMGGM	236	11	625
YQGSYGRLGF	103	11	626

Table XX
p53 A01 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0101	SEQ ID NO.
AKSVTCTY	119	8	8	627
CTTHIYNY	229	8	8	628
GSDCTTHY	226	9	9	629
GSDCTTHIYNY	226	11	11	630
GTAKSVTCTY	117	10	10	631
GTRVRAMAIY	154	10	10	632
LSSVPSQKTY	93	11	11	633
NTERIISVVVPY	210	11	11	634
PSOKTYQGSY	98	10	10	635
RIISVVVPY	213	8	8	636
RVEGNLRVEY	196	10	10	637
SSSVPSQKTY	94	10	10	638
SSVPSQKTY	95	9	9	639
VGSDCTTHY	225	10	10	640
VPSOKTYQGSY	97	11	11	641

Table XVI
p53 Δ03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
AAPPVAPA	69	8		642
AAPPVAPAPA	69	10		643
AAPPVAPAPAA	69	11		644
AAPTAAAPA	78	9		645
AAPTAAAPAPA	78	11		646
ACTGRDRR	276	8		647
AGKEPGGSR	355	9	0.0006	648
AGKEPGGSRRA	355	10		649
AGKEPGGSRRAH	355	11		650
AIYKQSQH	161	8		651
ALELKDAQA	347	9		652
ALELKDAQAAGK	347	11	0.0012	653
ALNKMFCOLA	129	10		654
ALNKMFCOLAK	129	11	0.4400	655
AMAIYKOSQH	159	10		656
CACPGRDR	275	8	-0.0001	657
CACPGRDRR	275	9	0.0014	658
CMGGMNRR	242	8	0.0003	659
CSDSGLA	182	8		660
CTTHIYNY	229	8	0.4600	661
CTYSPALNK	124	9		662
CTYSPALNKMIF	124	11		663
DCTTHIYNY	228	9		664
DDNTRFRI	207	8		665
DGEVFTQIR	324	10	0.0001	666
DGLAPFQII	186	8		667
DGLAPPQHLIR	186	11		668
DSDGLAPPQH	184	10		669
DSSGNLLGR	259	9	0.0014	670
DSTPPPGTR	148	9	0.0014	671
DSTPPPGTRVR	148	11	-0.0009	672
EAAPPVAPA	68	9		673
EAAPPVAPAPA	68	11		674
EALCLKDA	346	8		675
EALCLKDAQA	346	10		676
EAPRMPEA	62	8		677
EAPRMPEAA	62	9		678
EDTGPDEA	56	8		679
EDPGRDEAPR	56	10	0.0001	680
EDSGNLLGR	258	10	0.0001	681
EGNLRVEY	198	8		682
ELKDAQAGK	349	9	0.0005	683
ELNEALELK	343	9	0.0220	684
ELNEALELKDA	343	11		685
ELPPGSKT	298	8	-0.0004	686
ELPPGSKTR	298	9	0.0002	687
ELPPGSKTRA	298	10		688
EMFRELNEA	339	9		689
ETFSDIWK	17	8	-0.0001	690
EVGSDCTTHI	224	10		691

Table XVI
p53 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
EVGSDCTTHY	224	11		692
EVVVCACFGR	271	10	0.0002	693
EVVRCRPH	171	8		694
EVVRCRPHH	171	9		695
EVVRCRPHIER	171	11	0.0017	696
FLJISGTAK	113	8	0.0130	697
FTEDPGDEA	54	10		698
FTLOIRGR	328	8	-0.0009	699
FTLOIRGRER	328	10	0.0006	700
FTLOIRGRERF	328	11		701
FLJISGTA	112	8		702
GFLISGTAK	112	9	0.0014	703
GPRLGFLH	108	8		704
GGRAHSSH	360	9		705
GGRAHSSHLK	360	11		706
GLAPQHLIR	187	10	0.0130	707
GSDCTTHH	226	8		708
GSDCTTHY	226	9	0.0010	709
GSDCTTHYNY	226	11		710
GSRAHSSH	361	8		711
GSRAHSSHLK	361	10	0.0003	712
GSYGRLGF	105	9		713
GSYGRFLGFLH	105	11		714
GTAKSVTCTY	117	10	0.0230	715
GTRVRA	154	8		716
GTRVRAAIY	154	10	0.0370	717
GTRVRAAIYK	154	11	1.1000	718
HLIRVEGNLR	193	10	0.0002	719
HMTEVVR	168	8	0.0046	720
HMTEVVRCPH	168	11		721
HSHLKS	365	8	-0.0001	722
HSHLKS	365	9	0.0014	723
KGOSTSRH	373	8		724
KGOSTSRHK	373	9	0.0014	725
KGOSTSRHK	373	10	0.0001	726
KMFCOLAK	132	8	0.3800	727
KSKKGOSTSR	370	10	0.0240	728
KSKKGOSTSRH	370	11		729
KSVICTYSPA	120	10		730
KTYOGSYGF	101	9		731
KTYOGSYGFR	101	10	2.6000	732
LAPQHLIR	188	9	0.0014	733
LDDNTER	206	8		734
LDDNTERH	206	9		735
LDGEYFTLOIR	323	11		736
LGFHISGTA	111	9		737
LGFHISGTAK	111	10	0.0001	738
LGRNSFEVR	265	9	0.0014	739
LIRVEGNLR	194	9	0.0005	740
LLGRNSFEVR	264	10	0.0002	741

Table XVI
p53 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
LSPDDIEQWF	45	10		742
LSPLPSQA	32	8		743
LSOETFDLWK	14	11		744
LSSSVPSOK	93	9	-0.0009	745
LSSSVPSOKTY	93	11	0.0014	746
MAIYKQSOH	160	9		747
MFRELNEA	340	8		748
MLSPDDIEQWF	44	11		749
MTEVVRRCPIH	169	10		750
MTEVVRRCPIHI	169	11		751
NLLGRNSF	263	8		752
NLLGRNSFEVR	263	11	-0.0009	753
NLRKKGEPIH	288	9		754
NLRKKGEPIH	288	10		755
NLRVEYLDLR	200	10		756
NSFEVRVCA	268	9	0.0002	757
NSSCMGGMNR	239	10		758
NSSCMGGMNRR	239	11	0.0001	759
NTRHSVVVPY	210	11	0.0012	760
NTSSSPQPK	311	9		761
NTSSSPQPKK	311	10	0.0009	762
NTSSSPQPKK	311	11	0.0035	763
NVLSPLPSQA	30	10	-0.0009	764
PAAPTAA	77	8		765
PAAPTAA	77	10		766
PALNKMFCQLA	128	11		767
PAPAAPTAA	75	9		768
PAPAAPTAA	75	10		769
PDDIEQWF	47	8		770
PDEAPRMPEA	60	10		771
PDEAPRMPEAA	60	11		772
PGSRAHSSH	359	10		773
PGPDEAPR	58	8		774
PGTRVRAMA	153	9		775
PGTRVRAMAIY	153	11		776
PLSSSVPSQK	92	10		777
PSOKTYQGSY	98	10	0.0021	778
PTAAPATA	80	9	0.0003	779
PVAPAPAA	72	8		780
QAGKEPGGSR	354	10		781
QAGKEPGGSRA	354	11	0.0001	782
QGSYGERLGF	104	10		783
QIRGRERF	331	8		784
QIRGRERFEMF	331	11		785
QSOHIMTEVVR	165	10		786
QSOHIMTEVVR	165	11	0.0014	787
QSTSRHKH	375	8	-0.0009	788
QSTSRHKHKLMF	375	11	0.0004	789
RAHSIIILK	363	8	0.5500	790
RAHSIIILKSK	363	10	0.0001	791

Table XVI
p53 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
RAISSILKSKK	363	11	0.0270	792
RAMAIYKQSQH	158	11		793
RCSDSDGLA	181	9		794
RDRRTEENLR	280	11		795
RFEMFRELNEA	337	11		796
RGRRERFEMF	333	9	0.0008	797
RLGFLIISGTA	333	10		798
RLGFLIISGTAK	110	10		799
RMPEAAPTPVA	110	11	0.0430	800
RTEENLR	65	10		801
RTEENLRK	283	8	-0.0001	802
RTEENLRKK	283	9	0.0015	803
RVCACPR	273	10	3.3000	804
RVCACPRGRDR	273	8	0.3500	805
RVCACPRGRDRR	273	10	0.0140	806
RVEGNLRVEY	196	11	0.0290	807
RVEYLDDR	202	10	0.0014	808
RVEYLDDRNITF	202	8	-0.0004	809
RVRAMAIY	156	11		810
RVRAMAIYK	156	8	1.5000	811
SCMGMNR	241	9		812
SCMGMNR	241	8		813
SDCTTHIY	227	9	0.0001	814
SDCTTHIYNY	227	8		815
SDGLAPPQH	185	10		816
SDSDGLAPPQH	183	9		817
SFEVRVCA	269	11		818
SGNLLGRNSF	261	8		819
SGTAKSVTCTY	116	10		820
SSCMGMNR	116	11		821
SSCMGMNR	240	9		822
SSCMGMNR	240	10	0.0200	823
SSGNLLGR	260	8	0.0001	824
SSGNLLGRNSF	260	11	-0.0005	825
SSILKSKK	366	8	0.0005	826
SSIPQPKK	314	8	-0.0001	827
SSIPQPKK	313	8	-0.0001	828
SSSOPPKK	313	9	0.0014	829
SSSVFSQK	94	8	0.0005	830
SSSVFSQKTY	94	10	0.0003	831
SSVFSQKTY	95	9	0.0002	832
STPPGTR	149	8	-0.0001	833
STPPGTRVR	149	10	0.0002	834
STPPGTRVRA	149	11		835
STSRHKKLMF	376	10		836
STSRHKKLMFK	376	11	0.3100	837
SVEPLSQETF	9	11		838
SVFSQKTY	96	8		839
SVICTYSPA	121	9		840
TAKSVTCTY	118	9		841

Table XVI
p53 Δ03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
TCTYSPALNK	123	10	0.0056	842
TFHSVVVPY	211	10		843
TLQIRGRER	329	9	0.0002	844
TLQIRGRERF	329	10		845
TSRHKKLMF	377	9		846
TSRHKKLMFK	377	10	0.0500	847
TSSSQPK	312	8	-0.0001	848
TSSSQPKK	312	9	0.0014	849
TSSSQPKKK	312	10	0.0001	850
VAPAPAAFTPA	73	11		851
VCACPGRR	274	9	0.0014	852
VCACPGRRDR	274	10	0.0001	853
VDSPTPGTR	147	10	0.0001	854
VGSDCTTHI	225	9		855
VGSDCTTHY	225	10	0.0003	856
VLSPLPSQA	31	9		857
VTCTYSPA	122	8		858
VTCTYSPALNK	122	11	0.0700	859
VVRCPIII	172	8		860
VVRCPIIIER	172	10	0.0990	861
WFTEDPGDEA	53	11		862
WVDSTPPGTR	146	11	-0.0009	863
YFTLQIRGR	327	9		864
YFTLQIRGRER	327	11		865
YGFRLGFLII	107	9	0.0092	866
YLDNRNTE	205	8		867
YLDNRNTER	205	9		868
YLDNRNTERH	205	10	0.0006	869
YSPALNKMF	126	9		870

60727" 6285460

Table XVII
p53 A11 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
ACTGRDRR	276	8		871
AGKEPGSR	355	9	0.0001	872
AGKEPGSRAH	355	11		873
AIYKOSQH	161	8		874
ALELDAQAGK	347	11	0.0005	875
ALNKMFCOLAK	129	11	0.0420	876
AMAIYKOSQH	159	10		877
CACPGRDR	275	8	-0.0001	878
CACPGRDRR	275	9	0.0003	879
CMGGMNRR	242	8	0.0006	880
CNSSCMGGMNR	238	11		881
CTIIHINY	229	8		882
CTYSPALNK	124	9	1.1000	883
DCTIIHINY	228	9		884
DDNTERII	207	8		885
DOEYFTLOIR	324	10	0.0002	886
DGLAPPQH	186	8		887
DGLAPPQHILIR	186	11		888
DSDGLAPPQH	184	10		889
DSSGNLLGR	259	9	0.0001	890
DSTPPGTR	148	9	0.0001	891
DSTPPGTRVR	148	11	-0.0002	892
EDPGDEAPR	56	10	0.0002	893
EDSSGNLLGR	258	10	0.0002	894
EGNLRVEY	198	8		895
ELKDAQAGK	349	9	0.0001	896
ELNEALELK	343	9	0.0052	897
ELPPGSTK	298	8	-0.0003	898
ELPPGSTKR	298	9	0.0005	899
ENLRKKGEPIH	287	10		900
ENLRKKGEPIH	287	11		901
ETFSIDLWK	17	8	0.0050	902
EVGSDCTIIH	224	10		903
EVGSDCTIIH	224	11		904
EVRVCACPGR	271	10	0.0001	905
EVVRCPIH	171	8		906
EVVRCPIHH	171	9		907
EVVRCPIHHER	171	11	-0.0002	908
FLISGTAK	113	8	0.0005	909
FTLOIRGR	328	8	-0.0001	910
FTLOIRGRER	328	10	0.0002	911
GFLISGTAK	112	9	0.0001	912
GFLGFLH	108	8		913
GGRAIISSH	360	9		914
GGRAIISSHLK	360	11		915
GLAPPQHILIR	187	10	0.0006	916
GNLRYEYLDNR	199	11		917
GSDCTIIH	226	8		918
GSDCTIIH	226	9	0.0290	919
GSDCTIIHINY	226	11		920

66022T" 26285460

Table XVII
p53 All Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*101	SEQ ID NO.
GSRAIISII	361	8		921
GSRAIISILK	361	10	0.0002	922
GSYGFRLGFLH	105	11		923
GTAKSVTCTY	117	10	0.0490	924
GTRVRAMAIY	154	10	0.0002	925
GTRVRAMAIYK	154	11	0.3300	926
HLIRVEGNLR	193	10	0.0002	927
IMTEVVRR	168	8	0.0003	928
IMTEVVRRCPII	168	11		929
ISSIILKSK	365	8	0.0005	930
ISSIILKSKK	365	9	0.0008	931
KQOSTRII	373	8		932
KQOSTRIHK	373	9	0.0002	933
KQOSTRIHKK	373	10	0.0002	934
KMFQOLAK	132	8	0.3600	935
KSKKGOSTSR	370	10	0.0002	936
KSKKGOSTSRH	370	11		937
KTYQGSYGFR	101	10	0.8800	938
LAPQHLIR	188	9	0.0001	939
LDDRNTFR	206	8		940
LDDRNTFRH	206	9		941
LDGEYFTLQIR	323	11		942
LGFLHSGTAK	111	10	0.0002	943
LGRNSFEVR	265	9	0.0001	944
LIRVEGNLR	194	9	0.0005	945
LLGRNSFEVR	264	10	0.0001	946
LNEALEK	344	8		947
LNMFCOLAK	130	10	0.0034	948
LSQETFSDLWK	14	11	0.0470	949
LSSSVPSQK	93	9	0.0028	950
LSSSVPSOKTY	93	11		951
MAIYKQSQH	160	9		952
MTEVVRRCPII	169	10		953
MTEVVRRCPIIH	169	11		954
NLLGRNSFEVR	263	11		955
NLRKKGEPII	288	9	-0.0002	956
NLRKKGEPIIH	288	10		957
NLRVEYLDDR	200	10		958
NNTSSSPQPK	310	10	0.0001	959
NNTSSSPQPKK	310	11	0.0002	960
NSSCMGMNRR	239	10	0.0320	961
NSSCMGMNRR	239	11	0.0015	962
NTFRISVVVVPY	210	11		963
NTSSSPQPK	311	9	0.0950	964
NTSSSPQPKK	311	10	0.0540	965
NTSSSPQPKKK	311	11	-0.0002	966
PGGSRAIISII	359	10		967
PGPDEAPR	58	8		968
PGTRVRAMAIY	153	11		969
PLSSVPSQK	92	10	0.0002	970

Table XVII
p53 A11 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
PNTSSQPK	309	11		971
PSOKTYQGSY	98	10	0.0003	972
QAGKEPGSR	354	10	0.0002	973
QSQIMTEVVR	165	10	0.0002	974
QSQIMTEVVR	165	11	-0.0002	975
QSTSRHKK	375	8	0.0004	976
RAISSHLK	363	8	0.0071	977
RAISSHLKSK	363	10	0.0002	978
RAISSHLKSKK	363	11	0.0038	979
RAMAIYKOSQH	158	11		980
RDRTEENLR	280	11		981
RGRREFEMFR	333	10		982
RLGFLHSGTAK	110	11		983
RTEENLR	283	8	0.0011	984
RTEENLRK	283	9	0.0001	985
RTEENLRKK	283	10	-0.0001	986
RVCACFGR	273	8	0.0910	987
RVCACFGRDR	273	10	0.0490	988
RVCACFGRDRR	273	11	0.0110	989
RVEGNLRVEY	196	10	0.0290	990
RVEYLDDR	202	10	0.0020	991
RVRAMAIY	156	8	-0.0003	992
RVRAMAIYK	156	9	0.7300	993
SCMGGMNR	241	8		994
SCMGGMNR	241	9	0.0038	995
SDCTTIHY	227	8		996
SDCTTIHYN	227	10		997
SDGLAPQH	185	9		998
SDSDGLAPQH	183	11		999
SGTAKSVICTY	116	11		1000
SSCMGGMNR	240	9	1.4000	1001
SSCMGGMNR	240	10	0.0860	1002
SSGNLLGR	260	8	0.0017	1003
SSHLKSKK	366	8	0.0026	1004
SSPOPKKK	314	8	-0.0001	1005
SSSQPKK	313	8	0.0013	1006
SSSQPKKK	313	9	0.0006	1007
SSSVISQK	94	8	0.0010	1008
SSSVISQKTY	94	10	0.0001	1009
SSVPSQKTY	95	8	0.0003	1010
STPPPGTR	149	9	-0.0001	1011
STPPPGTRVR	149	10	0.0006	1012
STRHKKLMFK	376	11	0.1300	1013
SVPSQKTY	96	8		1014
TAKSVICTY	118	9	0.0120	1015
TCTYSPALNK	123	10		1016
TERISVVVPY	211	10		1017
TLQIRGR	329	9	0.0001	1018
TSRIKKLMFK	377	10	0.0052	1019
TSSSQPK	312	8	0.0019	1020

Table XVII
p53 A11 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*101	SEQ ID NO.
TSSSPQPKK	312	9	0.0001	1021
TSSSPQPKK	312	10	0.0002	1022
VCACTGRDR	274	9	0.0001	1023
VCACTGRDR	274	10	0.0002	1024
VDSPTPGTR	147	10	0.0002	1025
VGSDCTTH	225	9	0.0002	1026
VGSDCTTH	225	10	0.0003	1027
VTCTYSFALNK	122	11	0.1200	1028
VVRCPIHI	172	8	0.0017	1029
VVRCPIHI	172	10	-0.0002	1030
WVDSPTPGTR	146	11		1031
YFTLOIRGR	327	9		1032
YFTLOIRGR	327	11		1033
YGRUGFLH	107	9	0.2600	1034
YLDNRNTER	205	9	0.0005	1035
YLDNRNTERH	205	10		1036

660F2T" / 6285460
Table XVIII
p53 A24 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
CMGGMNRRPI	242	10		1037
CMGGMNRRPIL	242	11		1038
EMFRELNEAL	339	10		1039
EYLDNRNTF	204	9	0.0010	1040
GMNRRPIL	245	8		1041
GMNRRPILTI	245	10		1042
GMNRRPILTH	245	11		1043
LMLSPDDI	43	8	0.0023	1044
LMLSPDDIEQW	43	11	-0.0003	1045
LWKLLPENNVL	22	11	0.0001	1046
MFRELNEAL	340	9		1047
MFRELNEALEL	340	11	-0.0004	1048
RFEMFREL	337	8	0.0280	1049
SYGFRLGFL	106	9	0.0200	1050
TFSDLWKLL	18	8	0.0016	1051
TYQGSYGF	18	9	0.0010	1052
TYQGSYGFRL	102	8	0.1100	1053
TVSPALNKMF	125	10	0.1200	1054
		10	5.1000	1055
				1056

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Table XIX
pS3 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2=01	DR3	DR4=04	DR4=15	DR5=11	DR5=12	SEQ ID NO.
VTCTSPAL	AKSVCTCTSPALNKM	119								1057
LKDAQKE	ALELKDAQKEPGG	347								1058
VAPAPAPT	APPVAPAPAPTAA	70								1059
MPEAAPVA	APRMPEAAPVAPAP	63								1060
WPLSSVPS	APSWPLSSSVPSQKT	88								1061
IIHNYMCS	CTTHHNYMCSN	229								1062
YFTLQIRGR	DGEYFTLQIRGRERF	324								1063
LSPDIEQW	DLMLSPDIEQWFE	42								1064
VEPLSQET	DPSVEPLSQETFS	7								1065
LRVEYDDR	EGNLRVEYDDRNTF	198								1066
VLSPLSQA	ENNVLSPLSQAMDD	28								1067
LAKTCTVOL	FCQLAKTCTVOLWVD	134								1068
LWKLLPENN	FSDLWKLLPENNVL	19								1069
LGFLISGTA	GFRLGFLISGTAKSV	108								1070
VRAMAIKQ	GTRVRAMAIKQSQH	134								1071
PRGSTKRA	HHIHLPGSTKRALPN	296								1072
VVPYEPV	HSVVVPYEPVEVGS	214								1073
YMCNSSCMG	IIHNYMCSNMGGMN	233								1074
WFTEDFGPD	IEQWFTEDFGPDEAP	50								1075
LPNNTSSP	KRALPNNTSSSPQPK	305								1076
LHSGTAKSV	LGLHSGTAKSVTCT	111								1077
MFCQLAKTC	LNMFCQLAKTCVQ	130								1078
LPSQAMDDL	LPLPSQAMDDLMLS	32								1079
ITLEDSSGN	LTHLEDSSGNLLG	252								1080
MNRRLPITI	MGMNRRLPITITL	243								1081
VVRRCPHIE	MTEVVRRCPHIERCS	169								1082
LEKDAOAG	NEALEKDAOAGKEP	345								1083
LSPLSQAM	NNVLSPLSQAMDDL	29								1084
IEQWFTEDP	PDDIEQWFTEDFGPD	47								1085
VGSDCTTH	PPEVGSDCTTHVNY	222								1086
LWVDSTPP	PVQLWVDSTPPGTR	142								1087
FLISGTAKS	QLWVDSTPPGTRVR	144								1088
FEVRVCACP	RLGFLHSGTAKSVTC	110								1089
FRIISVVVY	RNSFEVRVCACPGRD	267								1090
LTITLED	RNTERHSVVVYEP	209								1091
ILITLED	RPILITLEDSSGN	249								1092
VRVCACGR	RRPILITLEDSSG	248								1093
LLGRNSFEV	SHEVRVCACGRDRR	269								1094
LNMFCOLA	SGNLGRNSFEVRVC	261								1095
MDDLMLSPD	SPALNMFCOLAKTC	127								1096
VFSOKTYQG	SQAMDDLMLSPDIE	37								1097
VPEPEV	SSSVFSOKTYQGSYG	94								1098
LSSSVPRGK	SVVVPYEPVEVGS	215								1099
FRJLGLHSG	SWPLSSSVFSOKTYQ	90								1100
WVDSTPPG	SYGFRJLGLHSGTAK	106								1101
YEPPEVGSID	VEYLLDRNTRFUSVV	203								1102
LPENNVLSP	VQLWVDSTPPGTRV	143								1103
	VVPYEPVEVGSIDCTT	217								1104
	WKLLENVNLSP	23								1105
	WKLLENVNLSP	234								1106

660121" 26285460

Table XIX
p53-DR Super-Nucleotide Binding Data

Core Sequence	Exemplary Sequence	DR6w192	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
VTCTSPAL	AKSVTCTYSPALNKM						1057
LKDAQAGKE	ALELKDAQACKEPGG						1058
VAPAPAAPT	APVPAPAPAAPTPAA						1059
MPEAAPVA	APRMEAPAPVAPAP						1060
WPLSSVPS	APSWPLSSSVPSQKT						1061
HIYNYMCS	CTIHIYNYMCSNCSM						1062
YFTLQIRGR	DGEYFTLQIRGRERF						1063
LSFDDIEQW	DLMLSPDDIEQWFE		-0.0018				1064
VEPLSQET	DPSVEPLSQETFS						1065
LRVEYLDNR	EGNLRVEYLDNRNTF						1066
VLSPLSOA	ENNVLSPLPSOAMDD						1067
LAKTCPVL	FCQLAKTCPVQLWVD						1068
LWKLIPENN	FSDLWKLIPENNVL						1069
LGFLHSCTA	GFRLGFLHSCTAKSV						1070
VRAMAIYKQ	GTRVRAMAIYKQSQH			1.7000			1071
LPFGSTKRA	HHLPFGSTKRALPN						1072
VVPYEPPEV	HSVVVYEPPEVGS						1073
YMCNCSMG	HIYNYMCSNCSMGGMN						1074
WFTEDPGD	IEQWFTEDPGDEAP						1075
LPNTSSP	KRALPNNTSSSPQPK						1076
LJSGTAKSV	LGFLHSCTAKSVTCT		-0.0007				1077
MFCOLAKTC	LNMFCOLAKTCPVQ						1078
LSQAMDDL	LSPLSQAMDDLMLS	0.0096	0.1500	0.0320			1079
ITLEDSSGN	LTITLEDSSGNLLG						1080
MNRRLTI	MGMNRRLTITL		-0.0007				1081
VVRRCPIHE	MTEVVRRCPIHERCS						1082
LELKDAQAG	NEALELKDAQAGKEP						1083
LSPLTSOAM	NNVLSPLTSOAMDDL						1084
IEQWFTEDP	PDDIEQWFTEDPGD						1085
VGSDCTTH	PVEGSDCTTHIYNY						1086
LWVDSPTTP	PVQLWVDSPTTPGTR						1087
VDSTPPGT	QLWVDSPTTPGTRVR						1088
FLHSGTAKS	RLGFLHSGTAKSVTC						1089
FEVRVCACP	RNSFEVRVCACPGRD						1090
FRISVVVY	RNTERHSVVVYEP						1091
LTITLED	RPILTITLEDSSGN						1092
ILTITLED	RPILTITLEDSSG						1093
VRVCACPR	SFEVRVCACPRDRR		0.0023				1094
LLGRNFEV	SGNLGRNFEVRVC						1095
LNKMFQCLA	SNLNKMFQCLAKTC						1096
MDDLMLSPD	SOAMDDLMLSPDDIE						1097
VPKTYOG	SSSVPSOKTYOGSYG						1098
VPYEPPEVG	SVVVPYEPPEVGSDC						1099
LSSSVPSOK	SWPLSSSVPSOKTYQ						1100
FRGLFHS	SYGFRGLFHSCTAK						1101
LDNRTRII	VEYLDNRTRHSVV						1102
WVDSPTTPG	VQLWVDSPTTPGTRV						1103
YEPPEVGS	VVYEPPEVGSCTT						1104
LPENNVLSP	WKLIPENNVLSP						1105
	WKLIPENNVLSP						1106

~~Table XXa~~

p53-DR-3A-Motif Peptides with Binding Data

Core	Exemplary	Position	DR3	DR4w4	DR4w15	DR5wH1	DR5wL2	SEQ ID NO.
Sequence	Sequence	Sequence						
LSPPDIEQW	DLMLSPDDIEQWFTE	42	0.0150					1107
LRVEYLDNR	EGNLRVEYLDDRNITF	198	0.0039					1108
LSQETFDL	EPPLSQETFDLWKL	11	-0.0025					1109
FTEDPGPDE	EQWFTEDPGPDEAPR	51	-0.0025					1110
LDGEYFTLQ	KKPLDGEYFTLQIRG	320	-0.0025					1111
ITLEDSSGN	LTHITLEDSSGNLLG	252	-0.0030					1112
LLPENNVL	LWKLLPENNVLPLP	22	0.0029					1113
VGSDCTTH	PIEVGSDCTTHINY	222	0.0380					1114
LWVDSTPP	PVQLWVDSTPPPGTR	142	0.0300					1115
IRVEGNLRV	QHLIRVEGNLRVEYL	192	0.0960					1116
MFRELNEAL	RFEFMRELNEATELK	337	0.0052					1117
YLDNRNTER	RVEYLDNRNTERHSV	202	0.1800					1118
YLDNRNTER	RVEYLDNRNTERHSV	215						1119
YLDNRNTER	RVEYLDNRNTERHSV	215						1120

090803Z JUL 71

Table X-Xb

p53-DR-3h-Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DICW281	DICW280	DICW15	DICW11	DICW12	DICW13
FTLQIRGE	GEYFTLQIRGERFE	325			0.0290			1120
VEGNLRVEY	LIRVEGNLRVEXLLDD	194			0.0930			1121
					0.0672			1122

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Table XXX

DR3-DR7 MHC Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw52	SEQ ID NO.
FTLQIRGRE	GEVETLQIRGREFE						1120
VEGNLRVEY	LIRVEGNLRVEYLD						1121
YKQSQHME	MAVYKQSQHMEVA						1122

TABLE XXI. Population coverage with combined HLA Supertypes

HLA-SUPERTYPES	PHENOTYPIC FREQUENCY					
	Caucasian	North American Black	Japanese	Chinese	Hispanic	Average
<u>a. Individual Supertypes</u>						
A2	45.8	39.0	42.4	45.9	43.0	43.2
A3	37.5	42.1	45.8	52.7	43.1	44.2
B7	38.6	52.7	48.8	35.5	47.1	44.7
A1	47.1	16.1	21.8	14.7	26.3	25.2
A24	23.9	38.9	58.6	40.1	38.3	40.0
B44	43.0	21.2	42.9	39.1	39.0	37.0
B27	28.4	26.1	13.3	13.9	35.3	23.4
B62	12.6	4.8	36.5	25.4	11.1	18.1
B58	10.0	25.1	1.6	9.0	5.9	10.3
<u>b. Combined Supertypes</u>						
A2, A3, B7	83.0	86.1	87.5	88.4	86.3	86.2
A2, A3, B7, A24, B44, A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, B58	99.9	99.6	100.0	99.8	99.9	99.8

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660727 " 6285460

Table XXXX A2 supermotif analogs

Source	AA	Sequence	A*0201 nM
p53.24	9	KLLPENNVL	313
p53.24V9	9	KLLPENNVV	385
p53.25	11	LLPENNVLSPL	19
p53.25V9	11	LLPENNVLSPV	39
p53.65	9	RMPEAAPPV	119
p53.65L2	9	RLPEAAPPV	78
p53.65	10	RMPEAAPVVA	78
p53.65L2V10	10	RLPEAAPPVV	143
p53.65M2V10	10	RMPEAAPPVV	54
p53.69	8	AAPPVAPA	5000
p53.69L2V8	8	ALPPVAPV	217
p53.101	11	KTYQGSYGFR	1786
p53.101L2V11	11	KLYQGSYGFRV	81
p53.113	11	FLHSGTAKSVT	5000
p53.113V11	11	FLHSGTAKSVV	1220
p53.129	9	ALNKMFCQL	735
p53.129V9	9	ALNKMFCQV	75
p53.129B7V9	9	ALNKMFBQV	192
p53.129	10	ALNKMFCQLA	1316
p53.129V10	10	ALNKMFCQLV	217
p53.132	9	KMFCQLAKT	333
p53.132V9	9	KMFCQLAKV	33
p53.132B4V9	9	KMFBQLAKV	125
p53.132L2V9	9	KLFCQLAKV	98
p53.135	9	CQLAKTCPV	208
p53.135L2	9	CLLAKTCPV	125
p53.135B1B7	9	BQLAKTBPV	102
p53.135B1L2B7	9	BLLAKTBPV	46
p53.139	9	KTCPVQLWV	725
p53.139L2	9	KLCPVQLWV	122
p53.139L2B3	9	KLBPVQLWV	46
p53.149	9	STPPPGTRV	909
p53.149M2	9	SMPPPGTRV	172
p53.149L2	9	SLPPPGTRV	122
p53.164	9	KQSQHMTEV	500
p53.164L2	9	KLSQHMTEV	122
p53.216	10	VVVPYEPPEV	617
p53.216L2	10	VLVPYEPPEV	89
p53.229	9	CTTIHYNM	278
p53.229L2V9	9	CLTIHYNV	263
p53.229B1L2V9	9	BLTIHYNV	116
p53.236	8	YMCNSSCM	4546
p53.236L2M8	8	YLCNSSCV	--
p53.236	11	YMCNSSCMGGM	667
p53.236L2M11	11	YLCNSSCMGGV	22
p53.255	11	ITLEDSSGNLL	1563
p53.255L2V11	11	ILLEDSSGNLV	33
p53.256	10	TLEDSSGNLL	1667
p53.256V10	10	TLEDSSGNLV	4167

650T2T 26285460

~~Table XXIIA A01 Analog Peptides~~

<u>Peptide</u>	<u>AA</u>	<u>Sequence</u>	<u>Source</u>	<u>A*0101 pM</u>
52.0136	11	GSDCTTIHYN	p53.226	67.6
57.0035	9	GTDCTTIH	p53.226.T2	0.9
57.0125	10	PTQKTYQGSY	p53.98.T2	35.7
57.0126	10	GTDKSVTCTY	p53.117.D3	42.4
57.0427	10	RVDGNLRVEY	p53.196.D3	45.5

660727 " 26285460

Table XXIIIB A03 Analog Peptides

Peptide	AA	Sequence	Source	A:0384 nM	A:1101 nM	A:2101 nM	A:3301 nM	A:6801 nM	A3-XRN
1371.14	10	KVYQGSYGFR	p53.101.V2	37.9	61.9	72	10000	40	4
1371.15	10	KVYQGSYGFK	p53.101.V2K10	33.3	9.2	138.5	-72500	38.1	4
1371.16	9	BVYSPALNK	p53.124.B1V2	15.7	12.8	439	22307.7	500	4
1371.17	9	BVYSPALNR	p53.124.B1V2R9	25	8.3	33.3	85.3	14.8	5
1371.18	8	KVFBQLAK	p53.132.V2B4	846.2	461.5	7500	-72500	8888.9	1
1371.2	11	GVRVRAMAIYK	p53.154.V2	57.9	136.4	418.6	-72500	13333.3	3
1371.22	9	RVRAMAIYR	p53.156.R9	40.7	1666.7	8.6	138.1	666.7	3
1371.24	9	SVBMGGMNK	p53.240.V2B3K9	12.5	17.1	9000	-72500	29.6	3
1371.25	10	SVBMGGMNRK	p53.240.V2B3K10	100	75	-36000	-72500	17	3
1371.26	9	SVBMGGMNR	p53.240.V2B3	161.8	95.2	120	852.9	11.1	4
1371.27	10	SVBMGGMNR	p53.240.V2B3	1000	25	620.7	805.6	11.4	2
1371.31	11	RVBABPGRDRK	p53.273.B3B5K11	314.3	200	4615.4	-72500	2500	2
1371.32	11	SVSRHKLMFK	p53.376.V2	33.3	54.5	295.1	18125	1509.4	3
1371.33	11	SVSRHKLMFK	p53.376.V2B4	196.4	2857.1	183.7	1384	598	3

650727" 26235160

Table XXII-A02-Analog Peptides.

Peptide	AA	Sequence	Source	A*0201-nM	A*0202-nM	A*0203-nM	A*0206-nM	A*6802-nM	A2-XRM
27.0068	9	KMFCQLAKT	p53.132	505.1	14.3	19.6	92.5	40000	3
39.0074	9	LLGRDSFEV	mp53.261	41.7					
44.0003	9	LLGRDSFEV	mp53.261	27.8					
1317.22	9	ALNKMFCQL	p53.129	735.3	390.9	18.5	72.5	-80000	3
1317.23	9	KMFCQLAKT	p53.132	333.3	33.1	17.5	105.7	-80000	4
1324.08	9	KQSQHMTVEV	p53.164	500	130.3	169.5	284.6	-80000	4
1329.04	9	CTTIHYNM	p53.229	277.8	286.7	2564.1	560.6	181.8	3
1329.07	9	KLLPENNVL	p53.24	312.5	1954.5	12500	1193.5	-80000	1
1338.08	10	FLHSGTAKSV	p53.113	357.1	170.2	14.5	4625	-80000	3

~~Table XXIID A24 Analog Peptides~~

<u>Peptide</u>	<u>AA</u>	<u>Sequence</u>	<u>Source</u>	<u>A*2401 nM</u>
52.008	8	TYQGSYGF	p53.102	109.1
52.0081	8	SYGFRLGF	p53.106	428.6
52.0103	10	TYQGSYGFRL	p53.102	100
52.0104	10	TYSPALNKMF	p53.125	2.4
52.0144	11	TYLWWWNNQSL	CEA.353	46.2
52.0147	11	TYLWWWVNGQSL	CEA.531	92.3
57.0042	9	LYWVNGQSF	CEA.533.Y2F9	15.8
57.0051	9	EYVNARHCF	Her2/neu.553.F9	150
57.007	9	TYSDLWKLF	p53.18.Y2F9	5.5
57.0071	9	SYGERLGFF	p53.106.F9	121.2
57.0090	10	TYQGSYGFRL	p53.102.F9	5.5

52.0103 " 52.0104

TABLE XXIII-B07-Analog Peptides-

Peptide	AA	Sequence	Source	B*0702-nM	B*3504-nM	B*5404-nM	B*5301-nM	B*5401-nM	BT-X033
48.0055	8	FPALNKMF	p53.127.F1	0.025	3000	18333.3	6200	3845.2	1
48.0234	11	FPALNKMFCQL	p53.127.F1	0.052	2482.8	5500	7750	500	2
48.0123	9	FPGTRVRAI	p53.152.F1	1.1	-36000	662.7	23250	2439	1
48.0196	10	FPPGSTKRAL	p53	0.79	-24000	6111.1	-23250	-20000	1
48.0127	9	FPGPKKKPI	p53	0.61	-36000	-55000	-31000	16666.7	1
48.0000	0	FPALNKMF	p53	0.025	3000	18333.3	6200	3845.2	1

650727" 26285460

Table XXIII: Immunogenicity of A2-Supermotif Peptides.

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound	CTL Peptide ¹	CTL Wild- type	CTL Tumor
p53.135	9	CQLAKTCPV	208	43.0	143.0	90.0	-- ²	4		1/4	0/4
p53.69	8	AAPPVAPA	5000	1536	1177	1233	4706	0			
p53.69L2V8	8	ALPPVAPV	217	7167	500	285	67	4	2/4	1/3	0/3
p53.129	9	ALNKMFCQL	735	391	19	73	-- ²	3			
p53.129V9	9	ALNKMFCQV	75	165	7.7	15	--	4	0/1		
p53.129B7V9	9	ALNKMFBQV	192	391	23	49	--	4	2/4	0/3	0/2
p53.132	9	KMFCQLAKT	333	33	18	106	--	4			
p53.132V9	9	KMFCQLAKV	33	8.4	7.7	15	--	4	1/3	0/2	0/2
p53.132B4V9	9	KMFBQLAKV	125	13	9.1	37	8889	4	5/5	0/4	0/4
p53.132L2V9	9	KLFCQLAKV	98	3.6	3.4	9.5	1270	4	2/3	1/3	0/3
p53.139	9	KTCPVQLWV	725	606	217	15	--	2			
p53.139L2	9	KLCPVQLWV	122	239	29	23	--	4	2/5	2/3	1/3
p53.139L2B3	9	KLBPVQLWV	45	29	19	31	--	4	3/4	2/3	1/2
p53.149	9	STPPPGTRV	909	1162	1031	--	129	1			
p53.149L2	9	SLPPPGTRV	122	226	13	9250	140	4	2/3	1/3	0/3
p53.149M2	9	SMPPPGTRV	172	215	13	425	667	4	2/4	2/4	2/4
p53.216	10	VVVPYEPPEV	617	1870	455	1194	--	1			
p53.216L2	10	VLVPYEPPEV	89	391	71	2056	--	3	1/1	1/1	
p53.255	11	ITLEDSSGNLL	1563	1265	2857	507	6667	0			
p53.255L2V14	11	ILLEDSSGNLV	33	123	71	206	--	4	1/3	0/3	0/2

¹Number of donors yielding a positive response/total tested.
²Indicates binding affinity $< 10,000$ nM.

650727 26285460

Table XXIV. MHC-peptide binding assays: cell lines and radiolabeled ligands.

A-Class I binding assays			Radiolabeled peptide		
Species	Antigen	Allele	Cell line	Source	Sequence
Human	A1	A*0101	Steinlin	Hu. J chain 102-110	YTAVVPLVY
	A2	A*0201	JY	HBVc 18-27 F6->Y	FLPSDYFPSV
	A2	A*0202	P815 (transfected)	HBVc 18-27 F6->Y	FLPSDYFPSV
	A2	A*0203		HBVc 18-27 F6->Y	FLPSDYFPSV
	A2	A*0206	FUN	HBVc 18-27 F6->Y	FLPSDYFPSV
	A2	A*0207	CLA	HBVc 18-27 F6->Y	FLPSDYFPSV
	A2	A*0207	721.221 (transfected)	HBVc 18-27 F6->Y	FLPSDYFPSV
	A3	GM3107		non-natural (A3CON1)	KVFPYALINK
	A11		BVR	non-natural (A3CON1)	KVFPYALINK
	A24	A*2402	KAS116	non-natural (A24CON1)	AYDNNYKIF
	A31	A*3101	SPACH	non-natural (A3CON1)	KVFPYALINK
	A33	A*3301	LWAGS	non-natural (A3CON1)	KVFPYALINK
	A28/68	A*6801	CIR	HBVc 141-151 T7->Y	STLPETYVVR
	A28/68	A*6802	AMAI	HBV pol 646-654 C4->A	FTQAGYPAL
	B7	B*0702	GM3107	A2 sigal seq. 5-13 (L7->Y)	APRTL VYLL
	B8	B*0801	Steinlin	HIV gp 586-593 Y1->F, Q5->Y	FLKDYQLL
	B27	B*2705	LG2	R 60s	FRYNGLIHR
	B35	B*3501	CIR, BVR	non-natural (B35CON2)	FPEKYAAAF
	B35	B*3502	TISI	non-natural (B35CON2)	FPEKYAAAF
	B35	B*3503	EHM	non-natural (B35CON2)	FPEKYAAAF
	B44	B*4403	PITOUT	EF-1 G6->Y	AEMGKYSFY
Mouse	B51		KAS116	non-natural (B35CON2)	FPEKYAAAF
	B53	B*5301	AMAI	non-natural (B35CON2)	FPEKYAAAF
	B54	B*5401	K43	non-natural (B35CON2)	FPEKYAAAF
	Cw4	Cw*0401	CIR	non-natural (C4CON1)	QYDDAVYKL
	Cw6	Cw*0602	721.221 transfected	non-natural (C6CON1)	YRHDGGNVL
	Cw7	Cw*0702	721.221 transfected	non-natural (C6CON1)	YRHDGGNVL
	D ^b		EL4	Adenovirus E1A P7->Y	SGPSNTYPEI
	K ^b		EL4	VSV NP 52-59	RGYVFQGL
	D ^d		P815	HIV-IIIIB ENV G4->Y	RGPYRAFVTI
	K ^d		P815	non-natural (KdCON1)	KFNPMKTYI
	L ^d		P815	HBVs 28-39	IPOSLSYWTSL

D-Class II binding assays				Radiolabeled peptide	
Species	Antigen	Allele	Cell line	Source	Sequence
Human	DR1	DRB1*0101	LG2	HA Y307-319	YPKYVKQNTLKLAT
	DR2	DRB1*1501	L466.1	MBP 88-102Y	VVHFFKNIVTPRTPPY
	DR2	DRB1*1601	L242.5	non-natural (760.16)	YAAFAAAKTAAFA
	DR3	DRB1*0301	MAT	MT 65kD Y3-13	YKTIAPDEEAR
	DR4w4	DRB1*0401	Preiss	non-natural (717.01)	YARFQSQTTLKQKT
	DR4w10	DRB1*0402	YAR	non-natural (717.10)	YARFQRQTTLKAAA
	DR4w14	DRB1*0404	BN 40	non-natural (717.01)	YARFQSQTTLKQKT
	DR4w15	DRB1*0405	KT3	non-natural (717.01)	YARFQSQTTLKQKT
	DR7	DRB1*0701	Pitout	Tet. tox. 830-843	QYIKANSKFIGITE
	DR8	DRB1*0802	OLL	Tet. tox. 830-843	QYIKANSKFIGITE
	DR8	DRB1*0803	LUY	Tet. tox. 830-843	QYIKANSKFIGITE
	DR9	DRB1*0901	HID	Tet. tox. 830-843	QYIKANSKFIGITE
	DR11	DRB1*1101	Swig	Tet. tox. 830-843	QYIKANSKFIGITE
	DR12	DRB1*1201	Herluf	unknown eluted peptide	EALHQLKINPYVLS
	DR13	DRB1*1302	H0301	Tet. tox. 830-843 S->A	QYIKANAKFIGITE
	DR51	DRB5*0101	GM3107 or L416.3	Tet. tox. 830-843	QYIKANAKFIGITE
	DR51	DRB5*0201	L255.1	Tet. tox. 830-843	PKYVKQNTLKLAT
	DR52	DRB3*0101	MAT	HA 307-319	NGQIGNDPNRDIL
	DR53	DRB4*0101	L257.6	Tet. tox. 830-843	YARFQSQTTLKQKT
	DQ3.1	A1*0301/DQB1*0	PF	non-natural (717.01)	YAHAAHAAHAAHAAHAA
Mouse	IA ^b		DB27.4	non-natural (ROIV)	YAHAAHAAHAAHAAHAA
	IA ^d		A20	non-natural (ROIV)	YAHAAHAAHAAHAAHAA
	IA ^e		CH-12	HEL 46-61	YNTDGGSTDYGLQNSR
	IA ^f		LS102.9	non-natural (ROIV)	YAHAAHAAHAAHAAHAA
	IA ^g		91.7	non-natural (ROIV)	YAHAAHAAHAAHAAHAA
	IE ^d		A20	Lambda repressor 12-26	YLEDARRKKAIYEKKK
	IE ^e		CH-12	Lambda repressor 12-26	YLEDARRKKAIYEKKK
	IE ^f				YLEDARRKKAIYEKKK

Table XXV. Antibodies used in MHC purification.

Monoclonal antibody	Specificity
W6/32	HLA-class I
B123.2	HLA-B and C
IVD12	HLA-DQ
LB3.1	HLA-DR
M1/42	H-2 class I
28-14-8S	H-2 Db and Ld
34-5-8S	H-2 Dd
B8-24-3	H-2 Kb
SF1-1.1.1	H-2 Kd
Y-3	H-2 Kb
10.3.6	H-2 IAk
14.4.4	H-2 IEd, IEK
MKD6	H-2 IAd
Y3JP	H-2 IAb, IAS, IAU

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Table XXVI. Crossbinding of A2 supermotif peptides

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound
p53.24	9	KLLPENNVL	313	1955	--	1194	--	1
p53.25	11	LLPENNVLSP	19	6.2	4.5	12	1702	4
p53.65	10	RMPEAAPVA	78	102	13	841	--	3
p53.65	9	RMPEAAPV	119	23	22	70	--	4
p53.113	10	FLHSGTAKSV	357	179	15	4625	--	3
p53.132	9	KMFCQLAKT	333	33	18	106	--	4
p53.135	9	QLAKTCPV	208	43	145	90	--	4
p53.136	8	QLAKTCPV	455	--	100	2643	1067	2
p53.164	9	KSQHMTVE	500	130	170	285	--	4
p53.187	11	GLAPPQHLRV	79	39	11	55	--	4
p53.193	11	HLIRVEGNLRV	385	1387	83	1194	1778	2
p53.229	9	CTTHYNYM	278	287	2564	561	181	3
p53.263	10	NLLGRNSFEV	217	--	2500	881	--	1
p53.264	9	LLGRNSFEV	85	358	37	206	--	4

-- indicates binding affinity $\leq 10,000$ nM

Table XXVII: Immunogenicity of A2-supermotif peptides.

Source	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles	CTL Wild- type ¹	CTL Tumor
p53.135	CQLAKTCPV	208	43	143	90	-- ²	4	1/4	0/1

1) Number of donors yielding a positive response/total tested.

~~2) Number of donors yielding a positive response/total tested.~~

660727" 26285460

~~Table XXVIII. Crossbinding of A2 supermotif analogs.~~

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound
p53.69	8	AAPPVAPA	5000	1536	1177	1233	4706	0
p53.69L2V8	8	ALPPVAPV	217	7167	500	285	67	4
p53.101	11	KTYQGSYGRL	1786	896	--	514	815	0
p53.101L2V11	11	KLYQGSYGFRV	81	48	24	116	--	4
p53.129	9	ALNKMFCQL	735	391	19	73	--	3
p53.129V9	9	ALNKMFCQV	75	165	7.7	16	--	4
p53.129B7V9	9	ALNKMFBQV	192	391	23	49	--	4
p53.129	10	ALNKMFCQLA	1316	1075	71	4625	--	1
p53.129V10	10	ALNKMFCQLV	217	287	71	7400	--	3
p53.132	9	KMFCQLAKT	333	33	18	106	--	4
p53.132V9	9	KMFCQLAKV	33	8.4	7.7	15	--	4
p53.132B4V9	9	KMFBQLAKV	125	13	9.1	37	8889	4
p53.132L2V9	9	KLFCQLAKV	98	3.6	3.4	10	1270	4
p53.135	9	CQLAKTCPV	208	43	143	90	--	4
p53.135L2	9	CLLAKTCPV	125	506	67	370	--	3
p53.135B1B7	9	BQLAKTBPV	102	71	15	67	--	4
p53.135B1L2B7	9	BLLAKTBPV	46	119	7.7	64	--	4
p53.139	9	KTCPVQLWV	725	606	217	15	--	2
p53.139L2	9	KLCPVQLWV	122	239	29	23	--	4
p53.139L2B3	9	KLBPVQLWV	46	29	19	31	--	4
p53.149	9	STPPPGTRV	909	1162	1031	--	129	1
p53.149M2	9	SMPPPGFRV	172	215	13	425	667	4
p53.149L2	9	SLPPPGTRV	122	226	13	9250	140	4
p53.164	9	KQSQHMTVEV	500	130	170	285	--	4
p53.164L2	9	KLSQHMTVEV	122	94	35	46	--	4
p53.216	10	VVPYEPPEV	617	1870	455	1194	--	1
p53.216L2	10	VLVPYEPPEV	89	391	71	2056	--	3
p53.236	11	YMCNSSCMGGM	667	391	67	974	5333	2
p53.236L2M1	11	YLCNSSCMGGV	22	13	3.6	18	1569	4
p53.255	11	ITLEDSSGNLL	1563	1265	2857	507	6667	0
p53.255L2V11	11	ILLEDSSGNLV	33	123	71	206	--	4

~~indicates binding affinity = 10,000nM.~~

Table XXXIX. Immunogenicity of A2-super-motif-analogs

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound	CTL Peptide ¹	CTL Wild- type	CTL Tumor
p53.69	8	AAPPVAPA	5000	1536	1177	1233	4706	0			
p53.69L2V8	8	ALPPVAPV	217	7167	500	285	67	4	2/4	1/3	0/3
p53.129	9	ALNKMFCQL	735	391	19	73	-- ²	3			
p53.129V9	9	ALNKMFCQV	75	165	7.7	15	--	4	0/1		
p53.129B7V9	9	ALNKMFBQV	192	391	23	49	--	4	2/4	0/3	0/2
p53.132	9	KMFCQLAKT	333	33	18	106	--	4			
p53.132V9	9	KMFCQLAKV	33	8.4	7.7	15	--	4	1/3	0/2	0/2
p53.132B4V9	9	KMFBQLAKV	125	13	9.1	37	8889	4	5/5	0/4	0/4
p53.132L2V9	9	KLFCQLAKV	98	3.6	3.4	9.5	1270	4	2/3	1/3	0/3
p53.139	9	KTCPVQLWV	725	606	217	15	--	2			
p53.139L2	9	KLCPVQLWV	122	239	29	23	--	4	2/5	2/3	1/3
p53.139L2B3	9	KLBPVQLWV	45	29	19	31	--	4	3/4	2/3	1/2
p53.149	9	STPPPGTRV	909	1162	1031	--	129	1			
p53.149L2	9	SLPPPGTRV	122	226	13	9250	140	4	2/3	1/3	0/3
p53.149M2	9	SMPPPGTRV	172	215	13	425	667	4	2/4	2/4	2/4
p53.216	10	VVPYEPPEV	617	1870	455	1194	--	1			
p53.216L2	10	VLYPYEPPEV	89	391	71	2056	--	3	1/1	1/1	
p53.255	11	ITLEDSSGNLL	1563	1265	2857	507	6667	0			
p53.255L2V11	11	ILLEDSSGNLV	33	123	71	206	--	4	1/3	0/3	0/2

1) Number of donors yielding a positive response/total tested.

2) -- indicates binding affinity < 10,000 nM.

Table XX. DR supertype primary binding

Peptide	DR147 Algo Sum	Sequence	Source	DR1 nM	DR4w4 nM	DR7 nM	DR147 Cross- binding
39.0307	2	GFRLGFLHSGTAKSV	p53.108	2.6	5.4	89	3
39.0308	2	LNKMFCQLAKTCPVQ	p53.130	20	804	167	3
39.0309	2	MGGMNRRPILTIHL	p53.243	--	--	--	0
39.0310	2	RRPILTIHLEDSSG	p53.248	5000	4500	--	0
39.0311	2	KRALPNNTSSSPQPK	p53.305	--	--	--	0
39.0312	3	DGEYFTLOIRGRERF	p53.324	125	--	--	1

-- indicates binding affinity = 10,000nM.

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Table XXV DR supertype cross-binding

Peptide	Sequence	Source	DR1 nM	DR4w4 nM	DR7 nM	DR2w2 DR1 nM	DR2w2 DR2 nM	DR6w1 9 nM	DR5w1 nM	DR8w1 nM	DR147 Binding	Broad Binding (5/8)
39.0307	GFRLGFLHSGTAKS	p53.108	2.6	54	89	253	167	76	100	29	3	8
39.0308	LNMFCQLAKTCPV	p53.130	20	804	167	5688	541	365	2500	1531	3	5

Table XXXII. DR3 binding

Peptide	Sequence	Source	DR3 nM
39.0409	EPPLSQETFSDLWKL	p53.11	--
39.0410	LWKLLPENNVLSPLE	p53.22	--
39.0411	DLMLSPDDIEQWFTE	p53.42	--
39.0412	EQWFTEDPGPDEAPR	p53.51	--
39.0413	PVQLWVDSTPPGTR	p53.142	--
39.0414	MAIYKQSQHMTEVVR	p53.160	--
39.0415	QHLIRVEGNLRVEYL	p53.192	3125
39.0416	LIRVEGNLRVEYLDD	p53.194	3226
39.0417	EGNLRVEYLDDRNTF	p53.198	--
39.0418	RVEYLDDRNTFRHSV	p53.202	1667
39.0419	SVVVPYEPPEVGSDC	p53.215	--
39.0420	PPEVGSDCTTIHYN	p53.222	7895
39.0421	LTITLEDSSGNLLG	p53.252	--
39.0422	KKPLDGEYFTLQIRG	p53.320	--
39.0423	GEYFTLQIRGRERFE	p53.325	--
39.0424	RFEMFRELNEALELK	p53.337	--

~~Indicates binding affinity = 10,000nM~~

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Table XXXIII. HFE candidate peptides

Peptide	Sequence	Source	DR1 nM	DR4w4 nM	DR7 nM	DR3 nM	DR2w2 01 nM	DR2w2 02 nM	DR6w1 01 nM	DR5w1 02 nM	DR8w1 01 nM	DR147 Binding	Broad Binding (5/8)	DR3 Binder
39.0307	GRLGFLHSCTAAEV	p53.108	2.6	5.4	89	--	253	167	76	100	29	3	8	0
39.0308	LNKMPQLAKTCPVQ	p53.130	20	804	167	--	5688	541	365	2500	1531	3	5	0

~~Peptide-binding affinity: 10⁻⁶ to 10⁻⁸ M~~
~~Peptide-binding affinity: 10⁻⁶ to 10⁻⁸ M~~

Chris 08/